Rust and FHB improvement of wheat through marker assisted selection and phenotyping

by

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Declaration

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Abstract

Wheat is one of the most widely cultivated crops of significant economic and nutritional importance. The predicted growth of the human population will result in higher food demands in the future. Wheat yield needs to sustainably increase to maintain food security. Unfortunately, the productivity of wheat are negatively impacted by abiotic and biotic stressors. Rust (leaf, stem and stripe) are the most prevalent disease on wheat that can cause severe damage to global production. Wheat rusts are caused by *Puccinia* spp. that are widely spread and highly adaptable. Fusarium head blight (FHB) on wheat is caused by *Fusarium* species that forms a complex that can produce mycotoxins in infected seeds. The FHB disease on wheat can result in reduction of seed quality and yield. One of the most important management factors to solve the problems posed by rust and FHB is by breeding for host plant resistance. Genetic resistance can be improved by using different rust and FHB resistance sources to achieve durable resistance.

The aim of this study was to generate molecular and phenotypic data of wheat lines to evaluate resistance to rust and FHB for improvement of genetic resistant material. An existing male sterile segregating MS-MARS population together with donor populations were screened for markers linked to rust and FHB resistance. Wheat lines that contained desirable traits were selected as parents in two breeding cycles of the male sterile mediated marker-assisted recurrent selection (MS-MARS) pre-breeding program. The donor lines were further evaluated by rust field phenotyping and FHB phenotyping under glasshouse conditions.

The molecular markers were successfully implemented to molecular characterise the populations and assisted in the parent selection process. The frequencies of two important slow rusting genes, *Sr2* and *Lr34,* were increased by 1.67% and 10.00%. The crossing cycles produced a large number of 3919 (cycle 1) and 9716 (cycle 2) hybrid seeds.

Rust field evaluations enabled identifying wheat lines that showed low susceptible phenotypic responses (20S to 40S) to leaf rust disease. Successful inoculations with *Fusarium graminearum* isolates were performed and disease severity between 13% and 100% were measured on 21 days post inoculation. The liquid

chromatography tandem mass spectrometry (LC-MS/MS) technique detected deoxynivalenol (DON) contamination levels above 0.032 ppb in fourteen wheat samples.

Future studies should aim at screening germplasms for the presence of more molecular markers linked to rust and FHB resistance. Selection of breeding parents should be based on both molecular data as well as phenotype data collected over multiple wheat growing seasons to improve effectiveness of resistance breeding.

Uittreksel

Koring is een van die mees wyd gekultiveerde gewasse wat belangrike ekonomiese en voedselwaarde het. Daar word voorspel dat die menslike populasie sal aanhou groei, wat sal lei tot verhoogde voedselbehoeftes in die toekoms. Koring opbrengste sal toenemend moet styg om voedselsekuriteit te verseker. Koring opbrengste word negatief beïnvloed deur biotiese en abiotiese stresfaktore. Roes (blaar, stam en streep) is een van die algemeenste siektes wat voorkom op koring en veroorsaak groot verliese aan wêreldwye produksie. Roes op koring word veroorsaak deur *Puccinia* spesies wat wyd verspreid voorkom en hoogs aanpasbaar is. Fusarium head blight (FHB) op koring word veroorsaak deur *Fusarium* spesies wat ‘n kompleks vorm en die vermoë het om mikotoksiene te produseer in geïnfekteerde sade. Koringteling vir weerstand teen roes en FHB is ‘n belangrike strategie om opbrengste te verhoog. Genetiese weerstand kan verbeter word deur verskillende roes en FHB weerstandsbronne te gebruik om duursame weerstand te bekom.

Die doel van die studie was om molekulêre en phenotipiese data van koringlyne te versamel om genetiese weerstand teenoor roes en FHB te evalueer en te verbeter. ‘n Populasie wat ontstaan het deur manlik steriele segregerende merker assisterend herhalende seleksie (MS-MARS), tesame met skenker populasies was ondersoek vir die teenwoordigheid van merkers wat geassosieer is met roes en FHB weerstand. Koringlyne, wat wenslike eienskappe besit, was gekies as ouers in twee telingsiklusse wat deel gevorm het van die MS-MARS voortelingsskema. Die skenkerlyne was verder ge-evalueer in ‘n roes phenotipering veldproef en FHB phenotipering glashuisproewe.

Die molekulêre merkers was suksesvol geïmplementeer om die populasies molekulêr te karakteriseer en het bygedra tot die ouer seleksie proses. Die geen frekwensies van die belangrike roesgene, *Sr2* en *Lr34*, het verhoog met 1.67% en 10.00%. Die kruising siklusse het ‘n groot aantal hibried sade van 3919 (siklus 1) en 9716 (siklus 2) geproduseer.

Evaluasies van roes in die veld het dit moontlik gemaak om koringlyne te identifiseer wat lae vatbare phenotipiese reaksies (20S tot 40S) getoon het teenoor blaarroes. Suksesvolle inokulasies met *Fusarium graminearum* isolate het plaasgevind en siekte ernstigheidsgrade van tussen 13% en 100%, was aangeteken

op 21 dae na inokulasie. Die 'liquid chromatography tandem mass spectrometry' (LC-MS/MS) tegniek was gebruik om deoxynivalenol (DON) kontaminasie vlakke bo 0.032 ppb te meet in 14 koringmonsters.

Die aantal molekulêre merkers wat geassosieer is met roes en FHB weerstand moet vermeerder word wanneer kiemplasmas in toekomstige studies ge-evalueer word. Seleksie en teling van ouers moet gebasseer word op beide molekulêre en phenotipiese data wat versamel word oor verskeie koring groeiseisoene om die effek van weerstandteling te verbeter.

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List of abbreviations

%	Percentage
°C	Degrees celsius
ABA	Abscisic acid
AFLP	Amplified fragment length polymorphism
APR	Adult plant resistance
APS	Ammonium persulphate
Avr	Avirulence
bp	Base pairs
CAF	Central analytical facility
CAPS	Cleaved amplified polymorphic sequence
C:I	Chloroform:isoamylalcohol
CIMMYT	International maize and wheat improvement center
cm	Centimetres
CTAB	Cetyltrimethylammoniumbromide
$CuSO_4$	Copper sulfate
CV	Correlation coefficient
DArT	Diversity arrays technology
dH_2O	Distilled water
DNA	Deoxyribonucleic Acid
DON	Deoxynivalenol
dpi	Days post inoculation
EMS	Ethyl methane sulphonate
EtBr	Ethidium Bromide

g	Gram
GA	Gibberellin acid
Gb	Gigabase pair
GCA	General combining ability
GxE	Genotype x environment interaction
GMS	Genetic male sterility
GS	Genomic Selection
GST	Glutathione S-transferase
EC	European Commission
EDTA	Ethylene-diamine-tetraacetic acid
FAO	Food and Agricultural Organization
FDK	Fusarium damaged kernels
FGSC	*Fusarium graminearum* species complex
FHB	Fusarium head blight
FRET	Fluorescence resonance energy transfer
f. sp.	Forma specialis
HPLC	High performance liquid chromatography
Hz	Hertz
IWGSC	International Wheat Genome Sequencing Consortium
JA	Jasmonic acid
KASP	Competitive allele specific PCR
kg	Kilogram
L	Litre
LC-MS/MS	Liquid chromatography tandem mass spectrometry

LTN	Leaf tip necrosis
Lr	Leaf rust resistance gene
LSD	Least significant difference
M	Intermediate (rust scoring)
M	Molar
MABC	Marker assisted backcrossing
MARS	Marker assisted recurrent selection
MAS	Marker assisted selection
MES	Makhathini Experimental Station
mL	Millilitre
mM	Micro molar
mm	Millimeteres
MR	Moderately resistant
MS	Moderately susceptible
MS-MARS	Male sterility mediated marker-assisted recurrent selection
NaCl	Sodium chloride
NaOH	Sodium hydroxide
ng/µL	Nanogram per microliter
NIV	Nivalenol
O	No visible infection
QTL	Quantitative trait loci
PAGE	Polyacrylamide gel- electrophoresis
PBC	Pseudo-black chaff
PCR	Polymerase chain reaction

PDA	Potato dextrose agar
PFT	Pore-forming toxin
Ppb	Parts per billion
pH	Potential of hydrogen
Pgt	*Puccinia graminis* f. sp. *tritici*
Pst	*Puccinia striiformis* f. sp. *tritici*
Pt	*Puccinia triticina*
R	Resistant
Rcf	Relative centrifugal force
RFLP	Restriction fragment length polymorphism
R-gene	Resistance gene
RH	Relative Humidity
Rht	Reduced height genes
RIL	Recombinant inbred lines
RMS	Recurrent mass selection
rpm	Revolutions per minute
S	Susceptible
SA	Salicylic acid
SCA	Specific combining ability
SCAR	Sequence characterized amplified region
SNP	Single nucleotide polymorphism
Sr	Stem rust resistance gene
SSR	Simple sequence repeat
SU-PBL	Stellenbosch University Plant Breeding Laboratory

Ta	Annealing temperature
TBE	Tris/Borate/EDTA
TCT's	Trichothecenes
TEMED	N, N, N‟, N‟-Tetramethylethylenediamine
Tris-Cl	Tris chloride
µL	Microliters
µM	Micrometre
USA	United States of America
UV	Ultra violet
V	Volts
v/v	Volume per volume
W	Watt
WAK2	Wall associated receptor-like kinase
WGS	Whole genome shotgun
WES	Welgevallen Experimental Station
w/v	Weight per volume
Yr	Stripe rust resistance gene
ZEA	Zearalenone
$ZnSO_4$	Zinc sulfate

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Chapter 1: Introduction

Bread wheat (*Triticum aestivum* L.) is considered a staple crop because it provides food for 40% of the world's population (Sinha and Shukla, 2017; Wang *et al.*, 2018). It is predicted that the human population will reach 9.8 billion by 2050 (Wang *et al.*, 2018). The Food and Agricultural Organization (FAO) estimated that wheat yields needs to increase with 38% or 0.86% annually to meet the demand of the growing population (Nalley *et al.*, 2018). From 2018 to 2019 South Africa needed to produce 3 million tons of wheat. South Africa only produced 1.9 million tons that resulted in importing of 1.4 million tons of wheat in 2018/19 (SAGIS, 2020). Genetic improvement of wheat is needed to significantly increase wheat yields to produce the required amount to feed the growing human population (Jia *et al.*, 2017).

Wheat has the ability to adapt to a range of climates, therefore making it one of the most widely cultivated crops (Jia *et al.*, 2017). The successful cultivation of wheat is negatively impacted by abiotic and biotic factors which significantly influences yield. Fungal diseases can be responsible for 15 - 20% of wheat yield losses per year. The prominent diseases of wheat includes rusts, blotches and head blight/scab that are contributors to these yield losses (Figueroa *et al*., 2018).

Wheat rusts are the most important biotic stressor that causes economic losses in South Africa (Figlan *et al.*, 2014). Leaf (*Puccinia triticina*) [Eriksson], stripe (*Puccinia striiformis* f. sp. *tritici*) [Eriksson] and stem (*Puccinia graminis* f. sp. *tritici*) [Eriksson & Henning] rust occurs on wheat. Sustainable increase of wheat production is significantly constrained by rust diseases (Savadi *et al.*, 2018). The virulent strain of stem rust Ug99 pathotypes TTKSF and PTKST has also been introduced to South Africa (Soko *et al.*, 2018). The long-term strategy to prevent yield losses caused by wheat rusts is by using varieties with genetic resistance which has proven to be economic, effective and protective (Hussain *et al.*, 2016). Rust resistance is broadly categorised as race-specific (controlled by major genes) or adult plant resistance (APR) (controlled by minor genes having a slow rust effect) (Aktar-Uz-Zaman *et al.*, 2017; Periyannan *et al.*, 2017). Rust pathogens have the ability to rapidly mutate, multiply and spread over long distances by using an air-borne dispersal mechanism

(Hussain *et al.*, 2016). New cultivars with durable resistance are needed because of the high adaptability of these pathogens (Dadrezaei *et al.*, 2013).

Wheat is also threatened by Fusarium head blight (FHB) which is caused by *Fusarium graminearum* [anamorph, Gibberella zeae Schwein (Petch)], *Fusarium culmorum* [W.G. Smith] and other *Fusarium* species (Miedaner *et al.*, 2018). Fusarium head blight causes significant yield losses due to shrivelled, light weight kernels (Dong *et al.*, 2018). The *Fusarium* species that causes FHB produce mycotoxins in infected grains (Miedaner *et al.*, 2018). When wheat is infested by FHB pathogens, mycotoxins such as deoxynivalenol (DON), DON derivatives, nivalenol (NIV) and zearalenone (ZEA) are produced in infected seeds. These mycotoxins are a severe threat to human and animal health which makes the grain unsuitable for animal feed and human consumption (Dong *et al.*, 2018; Yi *et al.*, 2018). Furthermore, 33 mycotoxin-producing *Fusarium* species have been identified in association with South African grain crops with *F. graminearum* being the pre-dominant species on South African wheat (Beukes *et al.*, 2018). The most effective method to control FHB is by using host resistance (Yi *et al.*, 2018). Fusarium head blight resistance is a complex trait, which is quantitatively inherited (Steiner *et al.*, 2017; Yi *et al.*, 2018). A total of 556 QTL's have been reported to contribute to FHB resistance in wheat with no single gene conferring complete resistance (Xiao *et al.*, 2016; Venske *et al.*, 2019).

The global breeding objective is to develop wheat cultivars with durable and effective resistance (Cristina *et al*., 2015). An ideal strategy is to pyramid different disease resistance genes such as rust and FHB resistance. This will reduce the loss of crop yield caused by multiple pathogens. The success of resistance gene pyramiding has been achieved by using marker-assisted selection (Zhang *et al.*, 2019). Marker assisted selection (MAS) is an indirect selection of a phenotype that takes place based on the genotype carrying desirable genes that could be detected through genetic markers (Gokidi *et al*., 2016).

MAS can effectively enhance breeding methods like recurrent mass selection when incorporated (Marais and Botes, 2009). Recurrent selection aims to increase the frequency of desirable alleles in a population (Gokidi *et al*., 2016). Recurrent selection involves selection, evaluation, recombination generation after generation and

repeating inter-mating between selected plants to produce the next cycle of selection of a heterozygous population (Gokidi *et al*., 2016). Recurrent selection is considered an effective strategy to improve a favourable polygenic trait while maintaining a high level of genetic variability (Wiersma *et al.*, 2001; Gokidi *et al*., 2016)

An effective method to accumulate several minor genes into a genetic background is by using recurrent selection strategies such as male sterility marker-assisted recurrent selection (MS-MARS). This breeding scheme makes use of the molecular markers, phenotypic measurements and the dominant male sterility (*Ms3*) gene to ensure cross-pollination of natural self-pollinating species. The MS-MARS breeding scheme can increase the level of durable resistance because there is continuous selection of wheat lines with favourable traits (Marais and Botes, 2009).

The aim of this study was to identify wheat lines resistant to wheat rust and FHB through MAS and phenotyping of wheat lines to improve the genetic resistant material. The selected wheat lines could then be included in MS-MARS pre-breeding program of the Stellenbosch University Plant Breeding Laboratory (SU-PBL). In order to accomplish the aim of the study, the following objectives were identified:

a) Molecular characterisation of pre-breeding germplasm developed at the SU-PBL. This entailed the molecular screening of a male sterile segregating population, the University of Stellenbosch's 2018 (F_{6}-generation) population and CIMMYT's 21FHBSN_015 nursery with a standard panel of markers.
b) Selected wheat lines with desirable genes and phenotypes were included as parents in cross-pollinations within the MS-MARS breeding scheme to improve breeding material.
c) Evaluating of selected MS-MARS lines in a rust phenotyping field trial for resistance against leaf and stem rust.
d) FHB phenotyping in the glasshouse of selected wheat lines. This entailed mimicking natural infection and FHB resistance was measured by disease incidence and severity and mycotoxin analyses.

Chapter 2: Literature review

2.1. Wheat

2.1.1. Importance of wheat production

Wheat is one of the most widely cultivated cereal crops with global production exceeding 765 million tonnes in 2017/18 (Wang *et al.*, 2018; Bhatta *et al.*, 2019). The top ten countries that contributes the most to the world wheat production are the following: China, India, Russia, United States of America (USA), France, Canada, Ukraine, Pakistan, Germany and Argentina as illustrated in Figure 2.1. (FAO: Countries production of wheat, 2019). From the global agricultural land 38.8% (more than 220 million hectares) of it is utilized for wheat production and it supplies 12-15% of protein per gram more compared to maize or rice (Abhinandan *et al.*, 2018; Balfourier *et al.*, 2019).

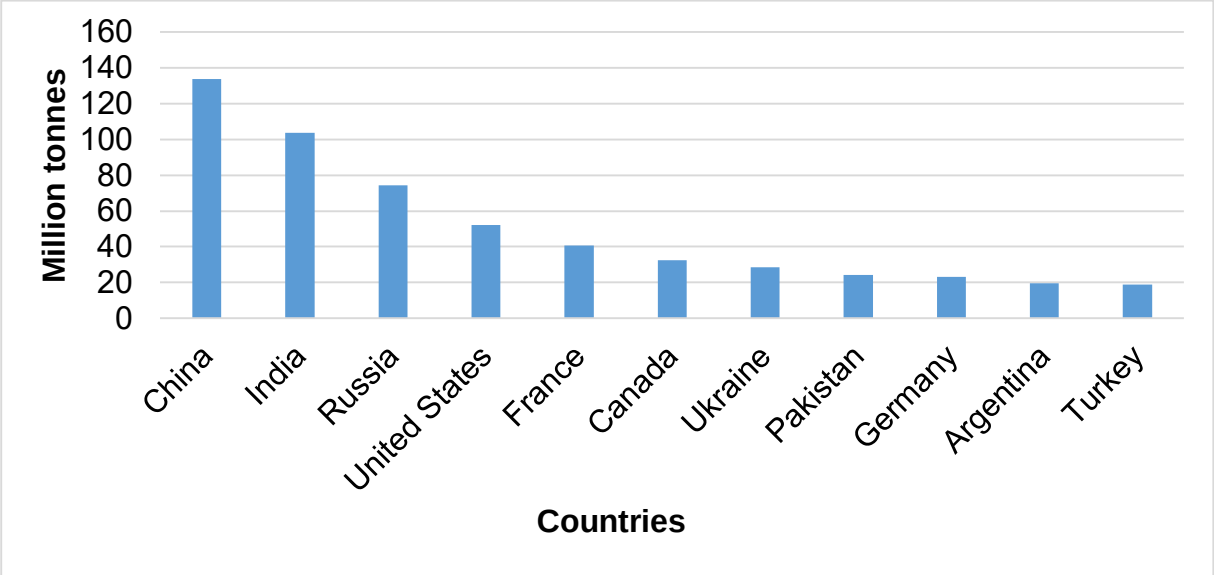

Figure 2.1. Top 10 wheat producing countries in 2019 (FAOSTAT, 2019).

Over the past 40 years world wheat production produced sufficient yields to maintain the balance between demand and supply. The prediction of human population growth and dietary changes will result in escalating demands and substantial yield grain will be needed over the next decades. The drive to increase

wheat quality and meet the yield demands also has other challenges such as lower genetic diversity of wheat because of breeding for elite high-performing cultivars and the decreasing of suitable farm land available (Figueroa *et al*., 2018). The reduction of arable and fertile land to grow crops like wheat, is expected to continue in the future (Wang *et al.*, 2018).

Studies have shown that the current wheat production rates do not meet the required targets (Wang *et al.*, 2018). In 2019 the average global wheat yield was 3 tonnes per hectare. It is estimated that the average yield needs to increase to 5 tonnes per hectare to meet the 2050 demands (Borisjuk *et al.*, 2019). The generally accepted solution to this problem is that the production rates need to increase, while still improving or maintaining its nutritional characteristics (Wang *et al.*, 2018; Borisjuk *et al.*, 2019).

2.1.2. Wheat production in South Africa

Bread wheat (*Triticum aestivum* L.) is considered a primary staple food, feeding more than one-third of the human population (Bhatta *et al.*, 2019). Wheat is one of the most consumed crops that provides 15-20% of energy needs and 25% of protein to the human diet (Balfourier *et al.*, 2019; Borisjuk *et al.*, 2019).

Wheat is the second most consumed grain crop behind maize in South Africa. It is estimated that South Africa's wheat consumption is 60.9 kg/year per capita (Nalley *et al.*, 2018). Wheat (bread wheat) is commercially cultivated in South Africa. Bread wheat is used in widely consumed food products such as breads, biscuits and noodles (Nhemachena and Kirsten, 2017).

Between 1999 and 2012 South Africa's wheat consumption have increased with an estimated of 8.9% (Nalley *et al.*, 2018). The country is also experiencing a decrease in the total production area and has been importing wheat since 1990 (Nalley *et al.*, 2018). South Africa's wheat planting area has decreased from a million hectares to 500 000 hectares in less than 20 years (Esterhuizen and Bonsu, 2019). South Africa is the second largest wheat producer in the Sub-Saharan Africa, behind Ethiopia. Food

security of the country could be helped by efficient increases of wheat production (Nalley *et al.*, 2018).

Wheat production of South Africa occurs in both winter and summer rainfall regions (Nhemachena and Kirsten, 2017). The main wheat producing provinces are Western Cape, Northern Cape and Free State, contributing 85% of South Africa's total wheat production. The Western Cape is a winter rainfall area that produces up to 50% (318 000 hectares) of South Africa's total wheat production (Esterhuizen and Bonsu, 2019). Dryland, spring wheat are grown in the Mediterranean climate of the Western Cape (Smit *et al.*, 2010). Spring wheat types are grown under irrigation in the summer rainfall areas and contributes 30% to South Africa's total wheat production (Nhemachena and Kirsten, 2017; Smit *et al.*, 2010). Winter or intermediate wheat are grown under dryland conditions in the Free State (Smit *et al.*, 2010).

2.1.3. The genetic background of wheat

Wheat was the first crop to be domesticated (Abhinandan *et al*., 2018) and consists of two types: the tetraploid durum wheat (*Triticum durum* L.) which accounts for 5% of the world's wheat and the hexaploid bread wheat which account for the other 95% (Peng *et al*., 2011b). The tetraploid durum wheat consists of two diploid genomes: AA and BB. The tetraploid durum wheat is mostly used for low-rising bread and pasta. The hexaploid bread wheat consists out of three diploid genomes namely the AA, BB and DD genomes which originated from two successive hybridization events (Figure 2.2.). The first polyploidization event occurred 0.5 million years ago between the wild diploid wheat *Triticum urartu* (AA genome) and an *Aegilops speltoides*-related specie (BB genome) (Peng *et al*., 2011b; Baidouri *et al.*, 2017). The hybridization resulted in the tetraploid *Triticum turgidum* spp. *diccocoides* (AABB genome). Molecular comparison studies at whole genome level showed that the B subgenome could be related to several *A. speltoides* lines (Baidouri *et al.*, 2017). The second polyploidization event occurred 10 000 years ago between the diploid goat grass *Aegilops tauschii* (containing the DD genome) and the cultivated tetraploid emmer wheat *T. turgidum* spp. *dicoccum* (AABB genome) (Brenchley *et al.*, 2012; Baidouri *et al.*, 2017). This spontaneous hybridization event resulted in the hexaploid bread wheat *T. aestivum* (AABBDD genome) (Baidouri *et al.*, 2017).

Cereal domestication primarily involved traits such as seed dormancy, seed development and spike morphology (Nave *et al.*, 2019). A natural mutation occurred about 8 500 years ago that changed the ears of the diploid goat grass (*Ae. tauschii*) and the cultivated emmer wheat (*T. dicoccum*) to a more easily threshed type. This change evolved into free-threshing ears that can both be seen on modern bread wheat and durum wheat (Peng *et al*., 2011b). Wild cereal spikes shatter at maturity that is known as brittle rachis (*br* gene trait) while domesticated cereals contains the non-brittle trait (Nave *et al.*, 2019). During the domestication and agronomic improvement of wheat modern breeding processes were applied and resulted in loss of genetic diversity. The genetic bottleneck resulted in an increase of wheat's vulnerability and susceptibility to environmental stresses such as pest and diseases (Peng *et al*., 2011b). It is estimated that during domestication the genetic diversity was reduced by 84% in durum wheat and 69% in bread wheat (Peng *et al*., 2011b).

The Green revolution contributed to the marked modifications in the wheat gene pool over the world (Balfourier *et al.*, 2019). The Green Revolution is known for the introduction of high yielding wheat varieties in combination with the large application of pesticides and fertilizer in the late 1960's (Hedden, 2003; Balfourier *et al.*, 2019). When high levels of fertilizer are applied to tall wheat plants it becomes susceptible to lodging. Wheat yield and quality was improved by reducing plant height by introducing dwarfing genes (*Rht*). The *Rht-1* homeoloci are dwarfing genes that are insensitive to the growth hormone gibberellic acid (GA). *Rht-D1* and *Rht-B1* genes encodes for DELLA proteins that represses growth. The presence of these genes resulted in an increase of grain production and decrease of straw production (Würschum *et al.*, 2017).

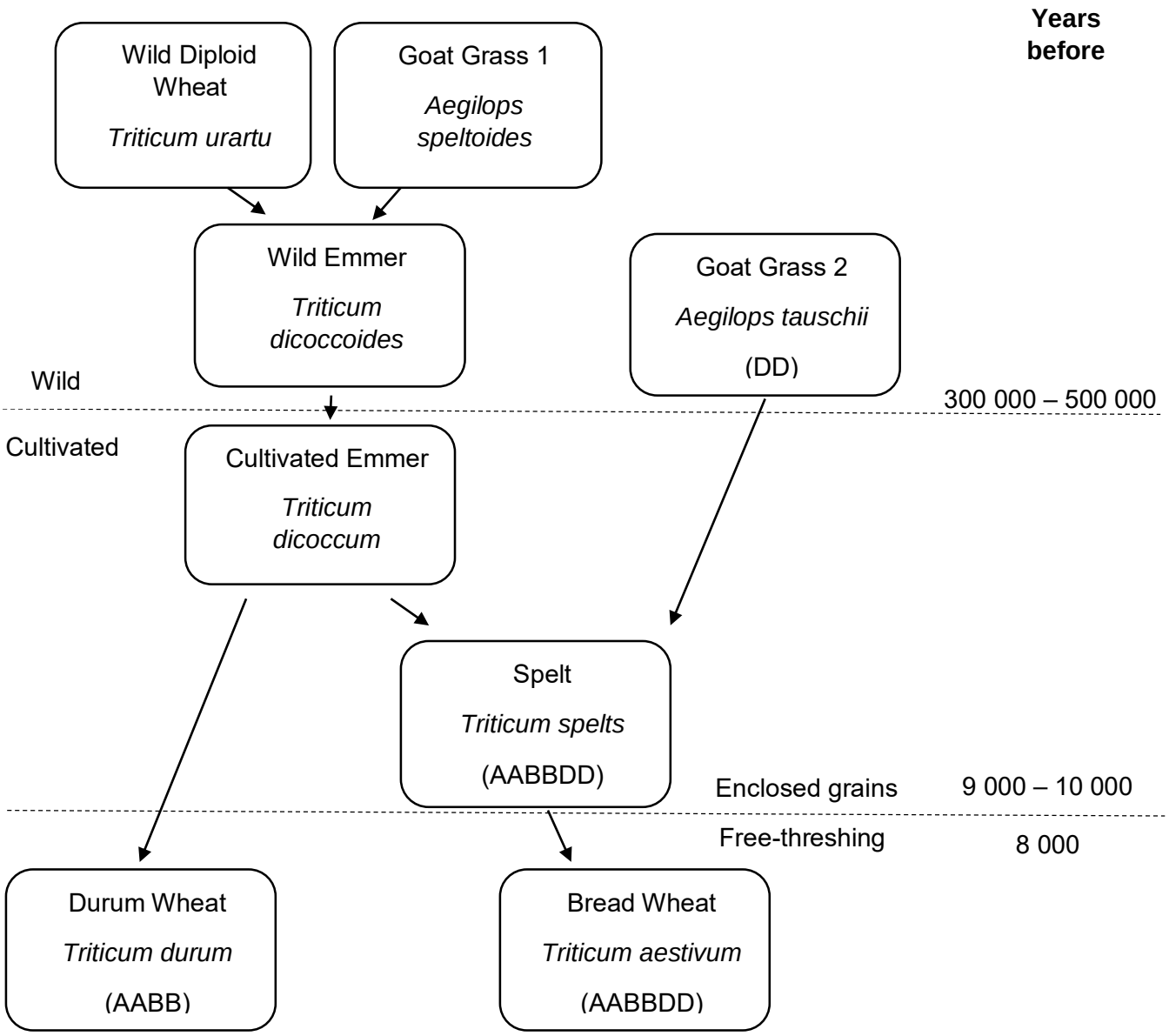

Figure 2.2. A diagram illustrating the domestication of bread wheat (*Triticum aestivum*) (Adapted from Peng *et al*., 2011a).

Bread wheat has a large complex genome of 17 gigabase pair (Gb) which contains 21 pairs of chromosomes (Tiwari *et al.*, 2016). The hexaploid wheat genome has a high percentage of 80-90% of repetitive sequences (Jia *et al.*, 2017). The first wheat genome sequence was published by Brenchley *et al.* (2012), who identified 132 000 single nucleotide polymorphisms (SNP's) in A (28.3%), B (29.2%) and D (33.8%) chromosomes. The assembly was highly fragmented, but provided to be a valuable tool to researchers serving as a framework for identifying genes, accelerate

further genome sequencing and to facilitate genome-scale analyses (Brenchley *et al.*, 2012; Borrill *et al*., 2015). In 2017 the first near-completed assembly of bread wheat's genome (Triticum 3.1) was published by Zimin *et al.* (2017). The assembly consisted out of a combination of very long and short sequence reads. The genome resembling 96% (close to 16Gb) of the whole genome, providing the most complete representation of 'Chinese Spring', but unfortunately it did not include gene annotation (Zimin *et al.*, 2017; Alonge *et al.*, 2020).

The International Wheat Genome Sequencing Consortium (IWGSC) published an annotated reference sequence in 2018 which represented the hexaploid wheat genome (IWGSC RefSeq v1.0) of the cultivar 'Chinese Spring'. The reference assembly missed a substantial portion of the genomic sequence because it was derived from short reads resulting in a more fragmented and less complete assembly compared to Triticum 3.1. Alonge *et al.* (2020) published an updated assembly (Triticum 4.0) that is 1.2 Gp bigger and identified 5700 new genes compared to the IWGSC assembly and annotation. The study was able to localize 97.9% of the sequence to chromosome revealing a more accurate representation of the 'Chinese Spring' repeat landscape. High quality wheat reference genome sequence contains updated genomics information that can be used in practical breeding programs.

2.1.4. Abiotic and biotic stressors

The productivity of wheat can be increased by reducing the losses caused by biotic and abiotic stresses (Abhinandan *et al.*, 2018). Environmental conditions or combinations thereof that negatively affects wheat's growth, development and reproduction is known as an abiotic stress. Wheat can be exposed to multiple diseases and pests because it is grown in a wide range of environments. The biotic stresses of wheat can be divided into foliar and stem diseases (wheat rusts), soilborne diseases (cereal cyst nematode and crown rot), seed transmitted disease and pests (Bhatta *et al.*, 2019).

Global grain yield needs to increase sustainably even when wheat yield are negatively impacted by climate change, pests and diseases (Crespo-Herrera *et al.*, 2017). In order to attain food security climate change and its impacts on yields of crops

needs to be addressed (Wang *et al.*, 2018). To achieve higher production under stressful environmental conditions is one of the most challenging propositions. It is predicted that with every 2°C increase in temperature wheat production will decrease with 6% (Abhinandan *et al.*, 2018). Cereal yield can be influenced by climate change that causes heat and water stresses. Climate change can also affect the impact of pathogens, pests and fertilizer supply (Wang *et al.*, 2018).

Climate change is a relevant factor because it can narrow or widen the range of pathogens. Areas that currently escape certain disease because of unfavourable environmental conditions could become favourable environments (Helfer, 2014). It is predicted that a warmer climate will result in more days with suitable temperatures for sporulation and more spores will be produced in an infected field. Conditions that are more dry and turbulent will result in a higher rate of spore emissions escaping to the free atmosphere (Prank *et al.*, 2019).

2.2. Rust diseases on wheat

Rust is the most prevalent disease on wheat and have been reported in all wheat growing regions worldwide (Limbalkar *et al.*, 2018). The economic important rust diseases on wheat are *Puccinia triticina* (*Pt*) (leaf rust), *Puccinia striiformis* f. sp. *tritici* (*Pst*) (stripe rust) and *Puccinia graminis* f. sp. *tritici* (*Pgt*) (stem rust) (Zhang *et al.*, 2019). These rust diseases can cause severe damage to the global wheat production. The estimated losses caused by these rust diseases worldwide ranged between 20-30 million tonnes in 2018 (Limbalkar *et al.*, 2018). The annual losses caused by wheat rust pathogens are estimated to be 4.3-5.0 billion US dollars (Figueroa *et al*., 2018).

Rust fungi are classified as obligate biotrophic parasites which requires living hosts to complete its life cycle and are dependent on extraction of nutritional resources from living host cells (Kolmer, 2013; Figueroa *et al*., 2018). Rust pathogens forms part of the Basidiomycete family and are members of the genus *Puccinia* (Figueroa *et al*., 2018). Rust fungi are adapted to a wide range of geographic areas, but most are highly specialized having a specific host range (Kolmer, 2013; Limbalkar *et al.*, 2018). The occurrence of rust disease on wheat are dependent on three critical elements: (1) the availability of susceptible host species; (2) both the life cycles of rust and its host plants

occurs in the appropriate time to have successful spore dispersal between the host plants and (3) to have favourable conditions during infection periods (Helfer, 2014).

The most relevant vector for rust diseases is the spread of urediniospores via atmospheric transport when environmental conditions are favourable (Helfer, 2014; Prank *et al.*, 2019). It has been confirmed that rust fungi spreads through atmospheric pathways from one continent to another, even crossings oceans (Prank *et al.*, 2019). Rust fungi have a high adaptability and evolutionary potential to evolve new virulent races that are easily dispersed (Helfer, 2014). When new virulent pathotypes of rust evolves it can cause yield losses from 50-90% in severely infected fields. In favourable environments severe epidemics of these rust diseases can occur (Limbalkar *et al.*, 2018).

The management strategies for wheat rust diseases involves crop husbandry, chemical and genetic control. Cultural control helps to manage the disease by removing alternative hosts and inter-crops 'green bridges' with tillage (Figueroa *et al*., 2018). Rust control is assisted by the absence of the alternative host *Berberis vulgaris* in South Africa (Ellis *et al.*, 2014). The damage caused by rust diseases are currently limited by applying fungicides when necessary (Limbalkar *et al.*, 2018). Fungicide treatments are weather dependant, costly and have some health and environmental concerns, therefore making genetic resistance the method of choice (Figueroa *et al*., 2018). The most sustainable and effective method to manage rust diseases is the development and deployment of resistant cultivars (Savadi *et al.*, 2018).

2.2.1. Life cycle of rust

Rusts are known to be heteroecious because two unrelated hosts are required to complete the rust pathogen's life cycle (Helfer, 2014). Rusts on cereals are macrocyclic and have five distinct stages involving: teliospores, basidiospores, urediniospores (occurs on cereal hosts), pycniospores and aeciospores (occurs on alternative hosts) (Figure 2.3.). The sexual cycle of *P. graminis* and *P. striiformis* are dependent on the presence of the common barberry (*B. vulgaris*) which act as a suitable alternative host. Urediniospores, basidiospores and aeciospores are often genetically highly diverse which results in the distinguishing between virulence and

avirulence of pathotypes on different host genotypes (Kolmer, 2013). Since *B. vulgaris* is absent in South Africa re-assortment of new resistance breaking combinations of avirulence (Avr) genes does not occur (Ellis *et al.*, 2014). Genetic diversity is introduced into South African *Pgt* populations through step-wise mutations and migration of other virulent pathotypes (Visser *et al.*, 2011).

The sexual part of the rust life cycle starts when haploid pycniospores are produced in flexous hyphae within pycnial structures. The dikaryotic nuclear division is restored by the fusion of two genetically distinct cells, known as plasmogamy (Kolmer, 2013). Two compatible pycniospores result in fertilization on the alternative host (Kolmer, 2013; Rodriguez-Algaba *et al.*, 2014). Insects often carry pycniospores to the other opposite mating type pycnial infections to result in fertilization. After successful fertilization aecium develops on the abaxial side of the leaves. On the inside of the dikaryotic aecium, aeciospores develops within chains (Rodriguez-Algaba *et al.*, 2014). Aeciospores are released, when the aecium has matured, and are dispersed by the wind to infect the primary grass host (cereal host). After infection of the cereal host uredinial infections develop (Kolmer, 2013).

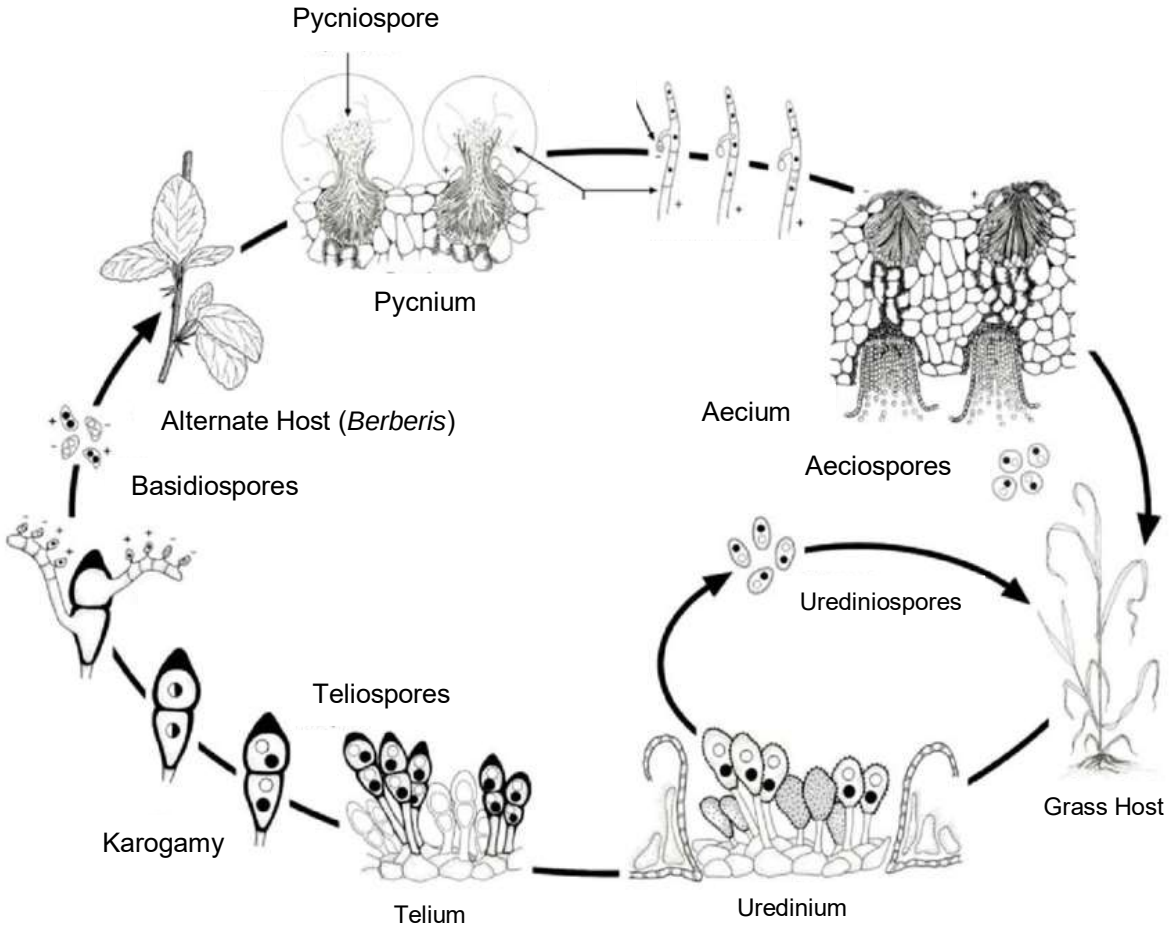

Figure 2.3. A diagram demonstrating the life cycle of *Puccinia striiformis* f. sp. *tritici* (Adapted from Kolmer, 2013).

On the cereal hosts the urediniospores have a dikaryotic nuclear condition and are 15 – 30 µm size in diameter (Kolmer, 2013; Helfer, 2014). Urediniospores are produced in vast numbers and in repeated short succession generations (Helfer, 2014). One uredinium can produce 10 000 spores per day under optimal conditions (less than 30°C) for 2-3 weeks (Prank *et al.*, 2019). This spore stage represents the rust epidemics on wheat and has the highest dispersal potential. Urediniospores has also shown to be the spread of new virulent rust pathotypes (Helfer, 2014). Merging of dikaryotic nuclei to form diploid nucleus (karyogamy) occurs in uredinal infections and teliospores develops (Kolmer, 2013; Rodriguez-Algaba *et al.*, 2014). Since rust is an obligate parasite it cannot survive on dead plant material, therefore resting teliospores are produced at the end of the wheat's growing season. Teliospores are

long-lived, dark-coloured, thick walled and resistant to UV radiation and desiccation (Helfer, 2014).

Binucleate haploid basidiospores emerges from the germinating diploid teliospores. The basidiospores develops (+) and (-) mating types after mitotic and meiotic divisions (Rodriguez-Algaba *et al.*, 2014). These basidiospores are ejected into the air and are normally wind dispersed (Kolmer, 2013; Helfer, 2014). In order for the basidiospores to infect it needs to land and germinate on the alternate host, *B. vulgaris* (barberry) (Kolmer, 2013; Rodriguez-Algaba *et al.*, 2014). After basidiospore infection pycnia forms and produces haploid pycniospores (Rodriguez-Algaba *et al.*, 2014). In the absence of *B. vulgaris* in South Africa the pathogen's asexual stage begins with the production of urediniospores on the wheat host. The uredinial stage on wheat persist throughout the year because it has the ability to move from winter wheat crops to spring and intermediate wheat crops (Figlan *et al.*, 2014).

2.2.2. Stripe rust

Winter cereal production across the world are being affected by stripe (yellow) rust disease and the causal agent is *Pst* (Chen *et al.*, 2014; Figueroa *et al*., 2018). Stripe rust can result in 100% of yield losses in susceptible cultivars making it the most economically important rust disease on wheat (Figueroa *et al*., 2018). This is because a cluster of uredinia are produced at a single urediniospore infection site and stripe rust infection is systemic (Chen, 2020). Yield losses between 10 and 70% caused by stripe rust disease occurs more commonly than 100% yield losses (Aktar-Uz-Zaman *et al.*, 2017). It was estimated that 88% of the world's wheat varieties are susceptible to stripe rust infection (Figueroa *et al*., 2018). Stripe rust was first reported in 1996 in South Africa (Boshoff *et al.*, 2002). Symptoms of stripe rust disease involves the appearance of mass yellow to orange urediniospores on susceptible plants (Figure 2.4.). These erupting pustules of urediniospores are arranged in narrow long stripes on leaves (usually between the veins), leaf sheaths, inner surfaces of glumes and lemmas of the head (Chen *et al.*, 2014). Wet and cool temperatures (between 7°C and 12°C) are favourable for this pathogen to cause disease (Figueroa *et al*., 2018; Chen, 2020).

Stripe rust disease has now adapted and spread to parts of the world with higher temperatures that were previously less affected which presents a threat to production regions of spring wheat (Aktar-Uz-Zaman *et al.*, 2017; Figueroa *et al*., 2018). In Australia, North America, Europe and South Africa the stripe rust populations appears to be clonal (Visser *et al.*, 2011; Figueroa *et al.*, 2018). The pathogen populations in Central Asia and Western China show a more significant level of genetic diversity. These polymorphic populations shows evidence of where sexual recombination occurs more often (Figueroa *et al*., 2018). Evidence from historical epidemics indicates that *Pst* is a serious threat to international wheat production (Chen *et al.*, 2014).

2.2.3. Leaf rust

Wheat leaf rust disease is caused by the pathogen *Pt* and causes yield losses by reducing kernel weight and the number of kernels per head (Figueroa *et al*., 2018). The leaf rust pathogen can cause losses of 50% and more on susceptible cultivars (Aktar-Uz-Zaman *et al.*, 2017). Earlier infection of Pt results in higher yield losses. Leaf rust symptoms on susceptible cultivars are seen on the leaf blades but also on leaf sheaths and glumes under high disease pressure. Yield losses of more than 30% can be accounted for when the infection on the plant ranges between 60-70% during the spike emergence and flag leaf stage (Draz *et al.*, 2015).

Wheat leaf rust coexist with wheat because of the pathogen's adaptability to a wide range of environments and climates (Aktar-Uz-Zaman *et al.*, 2017; Figueroa *et al*., 2018). Leaf rust is the most common rust disease impacting wheat production and favourable environmental conditions for this causal agent are temperatures between 10°C and 30°C. (Singh *et al.*, 2002; Figueroa *et al*., 2018). In most parts of the world where the disease occurs the pathogen's populations are clonal. The Fertile Crescent region of the middle East are known to be the centre of origin of leaf rust because of the presence of both the alternative and primary hosts. When Pt displays high genetic diversity it can result in the constant emergence of new virulent races (Figueroa *et al*., 2018).

Figure 2.4. Illustrating the different symptoms of rust on wheat. a) Leaf rust, b) Stripe rust and c) Stem rust. (Adapted from Kolmer, 2013; Figueroa *et al*., 2018).

2.2.4. Stem rust

Wheat stem (black) rust is caused by *Pgt*. Stem rust disease outbreaks have a negative impact on the wheat's yield by reducing the grain size and causing lodging of the plant. The disease development is favoured moist and warmer conditions (15°C-24°C) and its occurrence is less common than the other two rust diseases on wheat (Figueroa *et al*., 2018; Kosgey, 2019). Stem rust disease symptoms are recognized by red-brown masses of urediniospores found on stems, leaf sheaths, glumes and awns of plants that are susceptible (Figueroa *et al*., 2018) (Figure 2.4.). Stem rust infection interrupts the flow of nutrients in wheat stems, resulting in weak stems, prone to lodging, and small, shrivelled seeds (Turner, 2012). Aktar-Uz-Zaman *et al.* (2017) reported that Eastern and Central Europe recorded 20-30% yield losses caused by stem rust.

The emergence and the geographical spreading of the Ug99 (TTKSK) race threatened the global wheat production (Figueroa *et al*., 2018; Zhang *et al.*, 2019). The pathotype TTKSK was detected in Uganda in 1999 which later spread to neighbouring countries in Africa (Zhang *et al.*, 2019). The Ug99 race showed virulence to a broad spectrum of resistance genes and 80-90% of wheat varieties grown worldwide were

susceptible to this stem rust race (Soko *et al.*, 2018; Prank *et al.*, 2019). The race TTKSK showed virulence to most of the stem rust resistant genes (*Sr*), especially the widely used *Sr31* resistance gene (Soko *et al.*, 2018; Zhang *et al.*, 2019). Thirteen pathotypes belonging to the Ug99 lineage have been identified in a number of African countries (Table 1.1.) between 1998 and 2018 (Soko *et al.*, 2018).

The outbreak of the TKTTF race in Ethiopia during 2013 and 2014 caused up to 100% yield losses in susceptible wheat cultivars (Prank *et al.*, 2019). This was another outbreak with a broadly virulent pathotype which illustrates how wheat production is continuously threaten by stem rust evolving virulent races (Bhattacharya, 2017). The TKTTF race have been identified as an additional race of stem rust (not related to the Ug99 lineage) (Soko *et al.*, 2018). The TKTTF race has been reported in Europe, ending its rust free decades (Prank *et al.*, 2019). In Germany six races, including TKTTF, were detected in 2013 (Soko *et al.*, 2018). A highly virulence TTTTF race caused another epidemic in 2016 in Sicily, affecting several thousand hectares of bread and durum wheat. None of these races has yet spread to southern Africa while only the Ug99 races PTKST, TTKSF and TTKSF+*Sr9h* have been identified both in South Africa and Zimbabwe (Soko *et al.*, 2018).

Table 1.1. *Puccinia graminis* f. sp. *tritici* belonging to Ug99 lineage with avirulence and virulence status on discriminating resistance genes, identified in various countries (Adapted from Singh *et al*., 2015).

Race	**Common alias**	**Resistance genes and avirulence (A) or virulence (V) status**					**Confirmed countries (year detected)**
		Sr31	***Sr21***	***Sr24***	***Sr36***	***Sr9h***	
TTKSK	Ug99	V	V	A	A	A	Uganda (1998), Kenya (2001), Ethiopia (2003), Sudan (2006), Yemen (2006), Iran (2007), Tanzania (2009), Eritrea (2012), Rwanda (2014), Egypt (2014)
TTKSF		A	V	A	A	A	South Africa (2000), Zimbabwe (2009), Uganda (2012)
TTKST	Ug99+*Sr24*	V	V	V	A	A	Kenya (2006), Tanzania (2009), Ethiopia (2010), Uganda (2012)
TTTSK	Ug99+*Sr36*	V	V	A	V	A	Kenya (2007), Tanzania (2009), Ethiopia (2010), Uganda (2012), Rwanda (2014)
TTKSP		A	V	V	A	A	South Africa (2007)
PTKSK		V	A	A	A	A	Kenya (2009), Ethiopia (2007), Yemen (2009)
PTKST		V	A	V	A	A	Ethiopia (2007), Kenya (2008), South Africa (2009), Eritrea (2010), Mozambique (2010), Zimbabwe (2010)
TTKSF+		A	V	A	A	V	South Africa (2010), Zimbabwe (2010)

2.3. Genetics of rust resistance

The interaction between wheat rust pathogens and its primary host generally follows the gene-for-gene model. This model is based on assuming that avirulence is dominant over virulence in the pathogen (Rodriguez-Algaba *et al.*, 2014). In this model the resistance of the host plant is dependent on the interaction between the pathogen avirulence (Avr) genes and the host resistance (R) genes (Periyannan *et al.*, 2017) (Figure 2.5.).

2.3.1. Race-specific resistance

Resistance to rust falls into two categories which is (i) all stage resistance (race-specific genetic resistance) and (ii) slow rusting, adult plant resistance (APR) (Ellis *et al.*, 2014; Rutkoski *et al.*, 2014).

All stage resistance includes race-specific genes (R-genes) that are involved in pathogen recognition. The efficacy of R-genes in the host is pathogen strain or race dependant. Broad spectrum resistance refers to R-genes that confer resistance to all races of a single pathogen species. New R-genes from a wild or domesticated relative species, not previously exposed to the specific pathogen, are introduced into a cultivated crop. This type of resistance is not considered durable because once the pathogen is introduced to this R-gene, strong selection occurs in the pathogen population and virulent races, previously avirulent, can evolve (Ellis *et al.*, 2014). This happens more frequently where major resistance genes has been identified, exploited and introduced into host plants on large agricultural scales (Helfer, 2014).

R-genes gives high levels of resistance with clear phenotypic effects making selection for rust resistance simple and economical. For R-genes to remain effective several genes with effective resistance against most of the rust races should be bred into one genetic background (gene pyramids or stacks). Multiple and independent mutations in different Avr genes are then required for a pathogen to become virulent or overcome the host resistance (Ellis *et al.*, 2014). In wheat many race-specific rust resistance genes have been genetically defined and with increasing numbers more are being cloned (Periyannan *et al.*, 2017).

2.3.2. Adult plant resistance

The expression of APR genes are characterized by slower pathogen growth (referred to as “slow rusting”) without necrotic responses (Ellis *et al.*, 2014). Adult plant resistance genes are only expressed in later stages (from stage 7 on the Feekes scale) of wheat development (Ellis *et al.*, 2014; Periyannan *et al.*, 2017). The resistance is described as quantitative resistance that is considered to be more durable than race specific resistance (Rutkoski *et al.*, 2014; Periyannan *et al.*, 2017). Slow rusting APR is not associated with hypersensitive responses and do not have an obvious immune response (Ellis *et al.*, 2014; Periyannan *et al.*, 2017). When individual undefined APR genes, that show varying levels of partial resistance, are combined in adult field growing plants, a “near immunity” can be achieved (Ellis *et al.*, 2014) corresponding to moderate resistance (MR) phenotype (0-30%).

Wheat breeders do selection for APR under field conditions. Effective selection of APR can be prevented when R-genes with stronger resistance phenotypes are present and mask the APR genes expression. Some APR genes are unlikely to be durable because the genes are pathotype specific (Ellis *et al.*, 2014). Non-race-specific APR genes are defined as resistance against all races of a pathogen species (Periyannan *et al.*, 2017). Combinations of both race specific and non-specific APR genes can result in adequate levels of APR in crops (Ellis *et al.*, 2014).

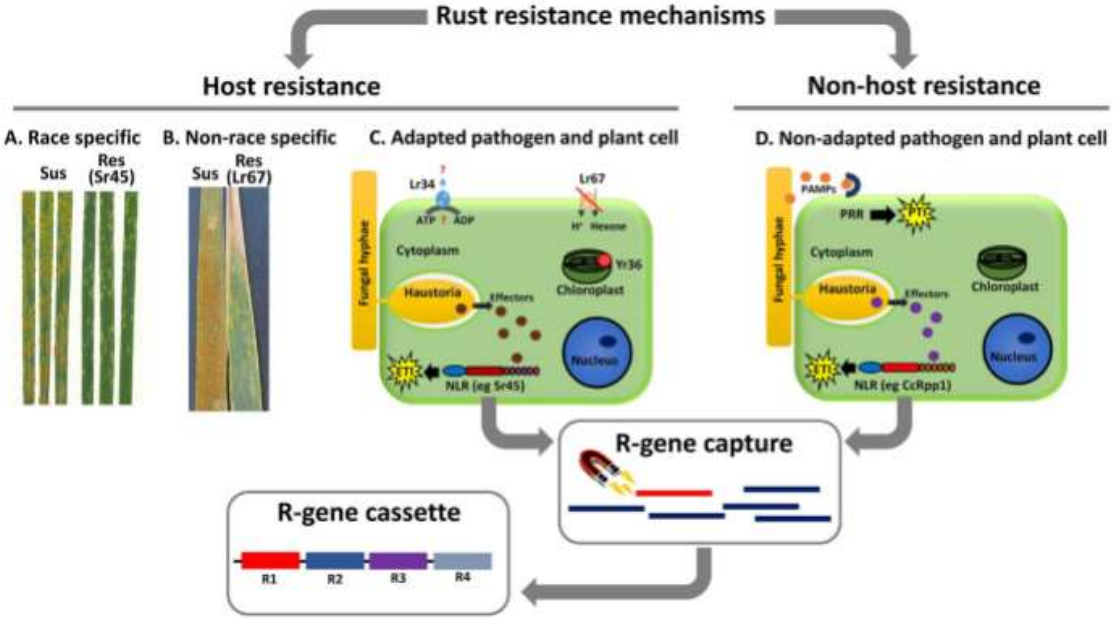

Figure 2.5. A diagram demonstrating the different rust resistance mechanisms between host resistance and non-host resistance (Adapted from Periyannan *et al*., 2017).

2.3.3. Rust resistance genes of importance to the current study

Wheat rust resistance genes are designated to *Yr, Sr* and *Lr* (stripe or yellow, stem and leaf rust resistance) (Ellis *et al.*, 2014). In 2017 more than 49 stripe rust, 58 stem rust and 80 leaf rust resistance genes have been reported. Most of these genes confers to race-specific resistance in diverse wheat or durum wheat cultivars and wild relative species(Aktar-Uz-Zaman *et al.*, 2017). To develop resistance to rust the construction of resistance gene cassettes are used. Resistance gene cassettes includes combinations of both APR and R-genes that are incorporate to be effective against rust species (Ellis *et al.*, 2014).

2.3.3.1. *Sr2/Yr30/Lr27/Pbc*

The adult plant rust resistance gene (*Sr2*) was introgressed into the hexaploid wheat when crosses were made with the cultivated emmer wheat cultivar 'Yaroslav' and the rust resistance cultivar 'Marquis' (hexaploid wheat). The initial crosses were made by an American breeder E.S. McFadden in 1961. After several selections for stem rust resistance, quality and yield in the lines H_{29} – H_{24} the variety 'Hope' was released. High levels of field resistance against *Pgt* races were observed in 'Hope' (Mago *et al.*, 2014). The *Sr2*-complex (*Sr2/Yr30/Lr27/Pbc*) was derived from the variety 'Hope' and confers to durable and broad spectrum resistance (Aktar-Uz-Zaman *et al.*, 2017).

Sr2 was mapped to the wheat chromosome arm 3BS (Ellis *et al.*, 2014). The gene confers to APR and is non race specific. *Sr2* resistance are characterized by its partial and non-hypersensitive resistance response with varying effectiveness under field conditions. The *Sr2* stem rust resistance is linked to a phenotypic trait pseudo-black chaff (PBC). This is a black-brown necrotic phenotype visible on the stem internodes and glumes. (Mago *et al.*, 2014). To detect the presence or absence of *Sr2* a cleaved amplified polymorphic sequence (CAPS) marker (csSr2) that is closely linked to the gene can be used (Mago *et al.*, 2011). Vishwakarma *et al.* (2019) reported using the microsatellite marker *Xgwm533* which is closely linked to the *Sr2* gene. The study reported significant allelic homoplasy at allele locus of *Xgwm533.* The marker can be used for rapid screening of large populations by using the SYBR green, low-resolution melt curve analysis.

The stem rust resistance gene *Sr2* is widely used with more than 50% of South Africa's wheat germplasms that are associated with this durable stem rust resistance gene (Ellis *et al.*, 2014; Aktar-Uz-Zaman *et al.*, 2017). For almost 100 years the *Sr2* gene has been effective against multiple pathotypes of stem rust in wheat growing regions (Aktar-Uz-Zaman *et al.*, 2017; Periyannan *et al.*, 2017).

For *Sr2* to be economically effective it must be combined with seedling resistance genes therefore providing effective stem rust resistance in wheat cultivars (Mago *et al.*, 2014). *Sr2* has the ability to boost the resistance levels of weak R-genes forming that is known as "*Sr2 complexes*" (Mundt, 2018). When selecting for high levels of stem rust resistance in the field, it often results in stacking *Sr2* with other

undefined APR genes and R-genes that are boosted by *Sr2.* If the R-genes are diverse these gene stacks contributes to varying levels of resistance durability. If the R-genes in the gene stacks do not provide strong resistance against all current races, the phenotype reverts to *Sr2* partial resistance response. To obtain adequate resistance the *Sr2* gene is mainly used in conjunction with other APR or race specific genes (Ellis *et al.*, 2014).

2.3.3.2. *Lr34/Sr57/Yr18/Pm38*

The gene *Lr34* was the first leaf rust resistance APR gene that was cloned and has been deployed extensively in commercial wheat varieties (Singh *et al.*, 2015). *Lr34* was mapped to wheat chromosome 7D and confers to partial resistance to leaf rust and is known for its long-term resistance against diverse pathotypes in different environments (Ellis *et al.*, 2014; Aktar-Uz-Zaman *et al.*, 2017)

The resistance gene causes reduction in haustorium formation and intercellular hyphal growth that results in slower infection development (Krattinger *et al.*, 2009). The *Lr34* gene encodes for a protein that is related to ATP-binding cassette (ABC transmembrane transporters) which the function of the resistance is still unknown (Ellis *et al.*, 2014; Periyannan *et al.*, 2017). The effective detection of the gene can be done by using the gene specific marker (cssfr1) and the co-dominant sequence tagged site marker (csLV34) in a multiplex reaction (Lagudah *et al.*, 2006; Krattinger *et al.*, 2009). During grain-filling stage *Lr34* are expressed in adult plants (Krattinger *et al.*, 2009). When *Lr34* is expressed it is associated with a leaf tip necrosis (LTN) phenotype and accelerated senescence that is most effective in the uppermost leaf (flag leaf) (Krattinger *et al.*, 2009; Ellis *et al.*, 2014; Periyannan *et al.*, 2017).

The gene was first described in 1966 in the cultivar ‘Frontana’ (Krattinger *et al.*, 2009). The *Lr34* gene is completely linked to partial stem rust resistance (*Sr57*), yellow rust resistance (*Yr18*) and powdery mildew resistance (*Pm38*), caused by the organism *Blumeria graminis* (Krattinger *et al.*, 2009; Ellis *et al.*, 2014). The stem rust resistance gene *Sr57* is effective or partially effective against the Ug99 lineage. The *Lr34* gene is known to be durable and capable of acting synergistically when combined with other

leaf rust resistance genes (Aktar-Uz-Zaman *et al.*, 2017). The effectiveness of R-genes is also enhanced or boost by the presence of *Lr34* (Ellis *et al.*, 2014). To achieve high levels of resistance the *Lr34* gene needs to be combined with other resistance genes into one genetic background (Krattinger *et al.*, 2009).

2.3.3.3. *Sr24/Lr24*

The *Sr24* gene confers resistance to stem rust in wheat and was spontaneous translocated from *Agropyron elongatum* chromosome 3AG to bread wheat's chromosome 3DL. The chromosome 3AG of *A. elongatum* also carried *Lr24* the leaf rust resistance gene. *Lr24* is closely associated with the red grain colour trait (Mago *et al.*, 2005).

The *Sr24* resistance gene functions to restrict pustule formation by causing necrosis (Mago *et al.*, 2005). The gene effectively conferred resistance to most of the *Pgt* races until it was overcome as a result of strong selection and rapidly evolving virulence to *Sr24* (Ellis *et al.*, 2014; Aktar-Uz-Zaman *et al.*, 2017). In South Africa virulence to the *Sr24* gene has also been reported. To detect the presence of the *Sr24* gene the amplified fragment length polymorphism (AFLP) marker Sr24#12 can be used (Mago *et al.*, 2005).

2.3.3.4. *Sr26*

The stem rust resistance gene *Sr26* was introgressed from *A. elongatum* chromosome arm 6A to hexaploid wheat's chromosome 6A. The Australian variety 'Eagle' was the first to carry the stem rust resistance *Sr26* gene and was released in 1971. The gene has been reported to be linked to a yield penalty thus limiting the use of the gene (Mago *et al.*, 2005). No virulence has been reported towards the *Sr26* segment and it remains effective against all *Pgt* races (Aktar-Uz-Zaman *et al.*, 2017). The advantages of using *Sr26* in the future include that: (i) It is still effective against Ug99, TTKST and TTTSK lineages, (ii) has good agronomical refined donor lines and (iii) it has a low frequency

among modern cultivars (Liu *et al.*, 2010; Aktar-Uz-Zaman *et al.*, 2017). To detect the presence of *Sr26* a combination of two dominant simple sequence repeat (SSR) markers Sr26#43 can be used that is associated with a 207bp band pattern (Mago *et al.*, 2005; Liu *et al.*, 2010).

2.3.3.5. *Sr31/Lr26/Yr9/Pm8*

Stem rust resistance was maintained in most countries by the common presence of *Sr31* in wheat varieties before the emergence of the Ug99 lineage (Ellis *et al.*, 2014). The *Sr31* gene was mapped to the short arm of chromosome 1 (1RS) along other race specific resistances (Mago *et al.*, 2005). The *Sr31* gene originated from the derived 'Petkus' rye. The *Sr31* was introgressed from 'Petkus" rye into bread wheat as a 1BL/1RS translocation (Das *et al*., 2006). Crosses between the winter wheat variety 'Kavkaz' and a Mexican spring semi-dwarf resulted in the 'Veery' varieties. CIMMYT developed the 'Veery' lines that contained the 1RS chromosome. The variety 'Kavkaz' carries the translocation chromosome 1RS that came from 'Petkus' rye (Mago *et al.*, 2005).

The detection of *Sr31* is done by using the sequence characterized amplified region (SCAR) markers iag95 (Mago *et al*., 2002). The gene cluster *Sr31/Lr26/Yr9/Pm8* confers resistance to race-specific stem rust, leaf rust, stripe rust and powdery mildew caused by *B. graminis* (formally known as *Erysiphe graminis*) (Mago *et al.*, 2002; Ben-David *et al.*, 2016). Enhanced resistance to stem rust can be achieved by combining for example *Sr31* and *Sr24* into one genetic background (Aktar-Uz-Zaman *et al.*, 2017).

2.3.3.6. *Lr37/Yr17/Sr38*

The *Lr37* gene confer to resistance to leaf rust in wheat (Helguera *et al.*, 2003). The *Lr37* gene is also linked to *Yr17* and *Sr38* conferring to leaf rust, stripe rust and stem rust resistance. These linked resistance genes were initially introgressed into 'VPM1'

winter bread wheat from *Triticum ventricosum* that was translocated from chromosome 2NS to wheat chromosome 2AS. The *Lr37* gene shows a certain level of resistance in the seedling growth stage at a temperature below 20°C. When the temperature rises above 20°C the gene becomes ineffective in seedlings and then acts as an APR gene (Bulos *et al*., 2006). In 2009 and 2010 the races 3SA145 (CCPS) and 3SA146 (MCDS) were identified with virulence against *Lr37* in South Africa. The race 3SA146 spread rapidly to major wheat growing areas. The *Lr37* gene are not considered effective in South Africa (Terefe *et al.*, 2014). The detection of this gene can be done by using the 2NS-specific primers VENTRIP-LN2 which were derived from restriction fragment length polymorphism (RFLP) probes (Helguera *et al.*, 2003).

2.3.3.7. Lr67/Yr46/Sr55/Pm46

The gene cluster *Lr67/Yr46/Sr55/Pm46* confers to leaf, yellow and stem rust as well as powdery mildew resistance (Figlan *et al.*, 2017). The cluster (*Lr67/Yr46*) was originally transferred from a Pakistani accession PI250413 to 'Thatcher' background, located on wheat's chromosome arm 4DL (Lagudah, 2011; Herrera-Foessel *et al.*, 2014). Developing SSR markers for the detection of the *Lr67* gene were inefficient (Forrest *et al.*, 2014). In a study done by Forrest *et al.* (2014) a single nucleotide polymorphism (SNP) based marker (csSNP856) associated with the stripe rust resistance gene *Lr67* was developed.

The leaf rust resistance gene *Lr67* is an APR gene which encodes for a protein that has lost its hexose transporter function. This results in a balance disturbance of sugars between the intracellular and extracellular spaces of the leaf. The change in the apoplastic sugar concentration may be involved in the activation of defence responses. This *Lr67* gene is effective against multiple biotrophic fungi by reducing the availability of nutrients inside the host cell (Periyannan *et al.*, 2017). The gene also cause LTN phenotype that is associated with accelerated senescence that can also be seen with the expression of the *Lr34* resistance gene (Aktar-Uz-Zaman *et al.*, 2017). Leaf tip necrosis is used to select for partial resistance to multiple pathogens as a morphological marker in breeding programs (Herrera-Foessel *et al.*, 2014).

2.3.3.8. Lr46/Yr29/Sr58/Pm39

The *Lr46* gene is referred to as a non-pathotype specific (APR) resistance gene that can boost various other strong or weak pathotype specific APR genes (Ellis *et al.*, 2014). The *Lr46* leaf rust resistance gene that provides slow or partial resistance is considered more durable than seedling resistance genes. The presence of this gene causes lower infection frequency, longer latent period, shorter period sporulation, small uredinia size and lower spore density (Aktar-Uz-Zaman *et al.*, 2017). It was reported that late field sowing associated with warmer temperatures can cause ineffectiveness of the *Lr46* gene (Lagudah, 2011).

In 1998 t*he Lr46* gene was identified in the wheat cultivar 'Pavon 76' on chromosome 1B (William *et al.*, 2003). In a study done by Cobo *et al.* (2018) a QTL *QYr.ucw-1BL* was found to be closely linked to the gene cluster but no marker is yet developed to detect the presence of these genes. The gene cluster (*Lr46/Yr29/Sr58/Pm39*) that confers to leaf, stripe and stem rust resistance as well as powdery mildew resistance is linked with the *Lr34/Yr18* cassette. The gene cluster has low LTN phenotypic expression in comparison to the *Lr34* gene (Lagudah, 2011). *Lr46* together with *Lr67* and *Lr34* confers to different levels of partial resistance while *Lr34* produce the strongest resistance (Ellis *et al.*, 2014). A small number of APR genes that confer to resistance against multiple diseases have been identified such as *Sr2/Yr30/Lr27/Pbc, Lr34/Sr57/Yr18/Pm38* and *Lr46/Yr29/Sr58/Pm39.* These accumulated genes results in partial resistance with additive gene effects. These gene clusters are considered to be very important for wheat breeding (Aktar-Uz-Zaman *et al.*, 2017; Crespo-Herrera *et al.*, 2017).

2.4. Rust phenotyping

Breeding and selecting for rust resistance includes performing routinely resistance phenotyping assays (Rahmatov *et al.*, 2019). To develop cultivars with a higher yield potential it is essential to make use of efficient phenotyping techniques (Velu and

Singh, 2014). Rust resistance disease response are characterise from no visual symptoms to small hypersensitive flecks to large masses of visible uredinia. The small hypersensitive flecks are surrounded by necrosis or chlorosis with restricted urediniospore production (Chen *et al.*, 2014). The epidemic status and host resistance expression in the field can be determined by doing appropriate rust scoring (Ali and Hodson, 2017).

Scoring of rust resistance is based on measuring field response (type of disease reaction) and disease severity (percentage of rust infection on plants). Disease severity is scored as a percentage following the modified Cobb scale. To measure the disease severity relies on visual observations that makes use of percentage infection intervals: 5, 10, 20, 40, 60 and 100. The field response is measured by making use of the following abbreviations: O, R, MR, M, MS and S (Table1.2.). Field response and disease severity measurements are combined in rust scoring for example 5MR would indicate that the plant showed a moderately resistant field response with 5% severity. Another example would be 60S suggesting the plant showed susceptible field response with 60% severity (Rust Scoring Guide – CIMMYT, 1986).

Table 1.2. The different field responses with associated designations in rust scoring.

Scoring	**Field response**
O	No visible infection on plants
Resistant (R)	Resistant - visible necrosis or chlorosis with no uredia present
Moderate resistant (MR)	Small uredia are present and are surrounded by either necrotic or chlorotic areas
Intermediate (M)	Variable sized uredia are present sometimes with necrosis or chlorosis or both
Moderately susceptible (MS)	Medium sized uredia are present and possibly surrounded with chlorotic areas
Susceptible (S)	Large uredia are present, generally with no necrosis or little to no chlorosis

2.5. Fusarium head blight of wheat

Wheat is threatened by Fusarium head blight (FHB) also known as scab, which is one of the most devastating diseases on wheat, causing reduction in yield and seed quality (Minnaar-Ontong, 2011; Dweba *et al.*, 2017a). Yield losses caused by FHB range between 30-40% and can increase up to 70% in susceptible cultivars and severe epidemics (He *et al*., 2013). The economic costs of FHB are shared by farmers, grain traders, millers and bakers that all forms part of the production chain. The economic losses experienced by farmers and the industrial sector results from the reduction in final yield and commercial quality parameters. It is estimated that the average costs of this disease on wheat in the USA is approximately 27 million US dollars which represents 3.7% of the annual value of the crop (Tayo *et al.*, 2018).

The FHB disease is a mature, floral plant disease of wheat which causes premature senescence of the wheat heads (Leplat *et al*., 2013; Figueroa *et al*., 2018). Several species that forms a fungal complex can cause FHB disease on wheat (Leplat *et al*., 2013). FHB is caused by fungi classified to the *Fusarium* genus (Góral *et al.*, 2019). The predominate specie in the FHB disease complex on wheat in many parts of the world was found to be *Fusarium graminearum* (Leplat *et al*., 2013) that forms part of the *Fusarium graminearum* species complex (FGSC). The fungal complex can consist out of more than 16 species that has a wide range of hosts. The predominant *Fusarium* species founded on small grain cereals in South Africa are *Fusarium graminearum, Fusarium culmorum and Fusarium avenaceum*. Other *Fusarium* species that are often associated with the disease are *Fusarium equiseti, Fusarium langsethiae, Fusarium poae, Fusarium sporotrichioides* and *Fusarium tricinctum* (Boutigny *et al.*, 2011). The geographical distribution of the FGSC shows that several of these species can co-exists (Tayo *et al*., 2018). It was reported that *F. graminearum* is the dominant specie of FHB on wheat in South Africa (Boutigny *et al.*, 2011).

The head of wheat plants gets infected via germination of deposited spores on or inside florets. The fungus infects the anthers and spreads throughout the rachis to other florets in the spikelet (Birr *et al.*, 2020). FHB fungal infection results in necrosis of spikelets, kernel damage and contamination of the grain and tissues with Fusarium toxins (Góral *et al.*, 2019). FHB disease leads to a reduction in the quality of the grain

by accumulation of mycotoxins which compromise the marketability of the grain (Figueroa *et al*., 2018). *Fusarium* species can produce toxins of different chemical groups. The most important chemical groups of small grain cereal contaminants are trichothecenes (deoxynivalenol (DON), nivalenol (NIV), T-2/HT-2 toxins), zearalenone (ZEA) and moniliformin that are produced in infected seeds (Dong *et al.*, 2018; Yi *et al.*, 2018; Góral *et al.*, 2019). These mycotoxins are a severe threat to human and animal health which makes the grain unsuitable for animal feed and human consumption (Dong *et al.*, 2018; Yi *et al.*, 2018). It is reported that DON is stable at 120°C and moderately stable at 180°C (Agriopoulou *et al.*, 2020).

The risks are increased by the stability of these mycotoxins in processed food and feed (Masri *et al.*, 2017). In grains and food products there are legally enforceable limits of these mycotoxins (Miedaner *et al.*, 2017). In 2017 the European Commission (EC) limits of DON and ZEA were 1250 µg kg^{-1} and 100 µg kg^{-1} for human consumption. The limits of Fusarium mycotoxins in animal feed were 8000 µg kg^{-1} DON and 2000 µg kg^{-1} ZEA (Edwards and Jennings, 2018). Recently, regulation regarding tolerances for fungus-producing toxins in foods has been amended in South Africa. The DON in grains are limited to 2000 µg kg^{-1} before processing and 1000 µg kg^{-1} after processing (Beukes *et al.*, 2018).

The mycotoxin DON that is produced by the fungus is known as a virulence or aggressiveness factor that helps the spread of infection from the wheat florets into the rachis and causes tissue necrosis (Mesterházy *et al.*, 2015). Deoxynivalenol and its derivatives are produced primarily by *F. graminearum* and *F. culmorum* (Wegulo, 2012). When DON is consumed by livestock it can lead to vomiting, food refusal, less effective feed utilisation and decrease in weight. When humans ingest DON-contaminated foods the effects have been associated with nausea, diarrhoea and vomiting (Beukes *et al.*, 2018). The mycotoxin NIV is considered to be more toxic than DON. Nivalenol has a similar structure and adverse effects on humans and animals than DON. The occurrence of DON in the field, food and feed products is far more commonly than the occurrence of NIV (Yazar and Omurtag, 2008). The *F. graminearum* species that produces NIV toxin has been less frequently associated with the grain of South Africa (Beukes *et al.*, 2018).

Residues and stubble from previous crops acts as reservoir for Fusarium inoculum (Ma *et al.*, 2020). Cultural practises such as burying plant residue material by deep ploughing could reduce the primary infections of the FHB pathogens. Inoculum accumulation are favoured by limited or no tillage (Ma *et al.*, 2020). The *F. graminearum* levels could significantly be reduced by crop rotation with potato, brassicas and legumes (Beukes *et al.*, 2018). Although there is chemical protection for this disease it only has a moderate effect. Studies have shown that fungicide applications are efficient to reduce FHB severity, but moderately resistant cultivars showed higher Fusarium mycotoxin DON contamination than susceptible cultivars. The most efficient fungicides could not keep the toxin level low under epidemic conditions (Buerstmayr *et al*., 2019). In South Africa no fungicides have been registered for the control of Fusarium disease on wheat (Beukes *et al.*, 2018). The most environmental friendly and economical method to solve the food hygiene and agricultural problems posed by FHB is by using host plant resistance (Niwa *et al.*, 2014). It is assumed that breeding for FHB resistance is the most effective method to also reduce DON contamination (Mesterházy *et al.*, 2015).

2.5.1. Disease cycle of FHB

Fusarium graminearum is considered a homothallic ascomycete. The fungus has both asexual and sexual life cycles with haploid mycelial structures that occurs in both cycles (Figure 2.6.). Homothallic or self-sterile heterothallic species forms mycelia through apomixes (Dweba *et al.*, 2017a). Three types of mitotic (asexual) spores are produces by mycelial structures in the asexual life cycle depending on the species: clamydospores are produced within or on hyphae, macroconidia are produced in sporodochia and microconidia are produced on conidiaphores (Dweba *et al.*, 2017a; Tayo *et al*., 2018). The fungus also produce ascospores in perithecia in the sexual life cycle (Dweba *et al.*, 2017a).

The primary inoculum of *F. graminearum* that causes FHB infection and development on wheat heads overwinters on crop residues. The primary inoculum consists out of airborne ascospores (sexual spores) and macroconidia (asexual spores) that are produced on mycelium growths on crop residues (Leplat *et al*., 2013). These spores are dispersed by wind or splash of rain (Kosgey, 2019). The

ascospores that are produced in perithecia can either be homozygous (selfed) or heterozygous (outcrossed). A higher genotypic diversity can be achieved when the outcrossing (heterozygous) event occurs. Higher genotypic diversity could result in natural populations that are able to adapt faster when it is expose to selective pressures such as fungicides and genetic resistance (Jenczmionka *et al*., 2003).

Fusarium head blight initial infection takes place during anthesis, in wheat florets, during the soft kernel development stage between 10-20 days (Jenczmionka *et al*., 2003). The ascospores and conidia spores lands on the floral tissue and can first colonize on the external surfaces of the glumes or directly infect through the stomata that exposes the anthers (Jenczmionka *et al*., 2003; Dweba *et al.*, 2017a; Tayo *et al*., 2018). After penetrating the anthers mycotoxins are produced which contaminates the grain (Dweba *et al.*, 2017a). Thereafter the floral ovary, floral bracts, lemma, palea and glumes gets penetrated (Jenczmionka *et al*., 2003). For FHB colonization to take place after inoculation the invasive mycelia spread into the rachial node and then up and down the rachis (Dweba *et al.*, 2017a). The pathogen spread from one floret to another via vascular bundles in the rachis of susceptible wheat plants (Kosgey, 2019). Visual symptoms develop after the fungi proliferate rapidly and spread intracellularly (Dweba *et al.*, 2017a). Bleaching and necrosis of wheat heads are the visual symptoms of FHB that leads to underdeveloped, shrivelled kernels, resulting in the reduction of yield (Jenczmionka *et al*., 2003; Dweba *et al.*, 2017a). The infected vascular tissue deprives grain from water and nutrients supply which leads to premature death of wheat heads (Kosgey, 2019).

In the asexual part of the life cycle macroconidia often initiate epidemics in inoculated fields (Kosgey, 2019). In the absence of *F. graminearum* hosts the fungi survives saprophytically on crop residues (Leplat *et al*., 2013). The fungus overwinters as spores on infested crop residues and in the soil (Leplat *et al*., 2013; Tayo *et al*., 2018). The fungus turns into an opportunistic destructive pathogen with a relatively short parasitic period and a narrow infection window (Buerstmayr *et al*., 2019).

The FHB infection and development are dependent on the vulnerable plant stage (spans over several days during anthesis), abundance of the inoculum, plant's resistance status and the environmental conditions during the critical infection period (Buerstmayr *et al*., 2019; Kosgey, 2019). Favourable environmental conditions for infections are warm temperatures that range between 20-25°C and prolonged periods of high humidity for 48-72 hours (McMullen et al., 2012). *Fusarium graminearum* (DON-producing) has an optimum infection temperature of 28°C but the minimum temperature for infection is lower (Suzuki *et al*., 2012). The mycelial growth of *F. graminearum* reaches an optimum at 25°C. Warm and humid conditions favours the germination and growth of conidia and ascospores (Leplat *et al*., 2013). In favourable environmental conditions and inoculum pressure, FHB can cause up to 70% yield losses in wheat (Zhang *et al*., 2011).

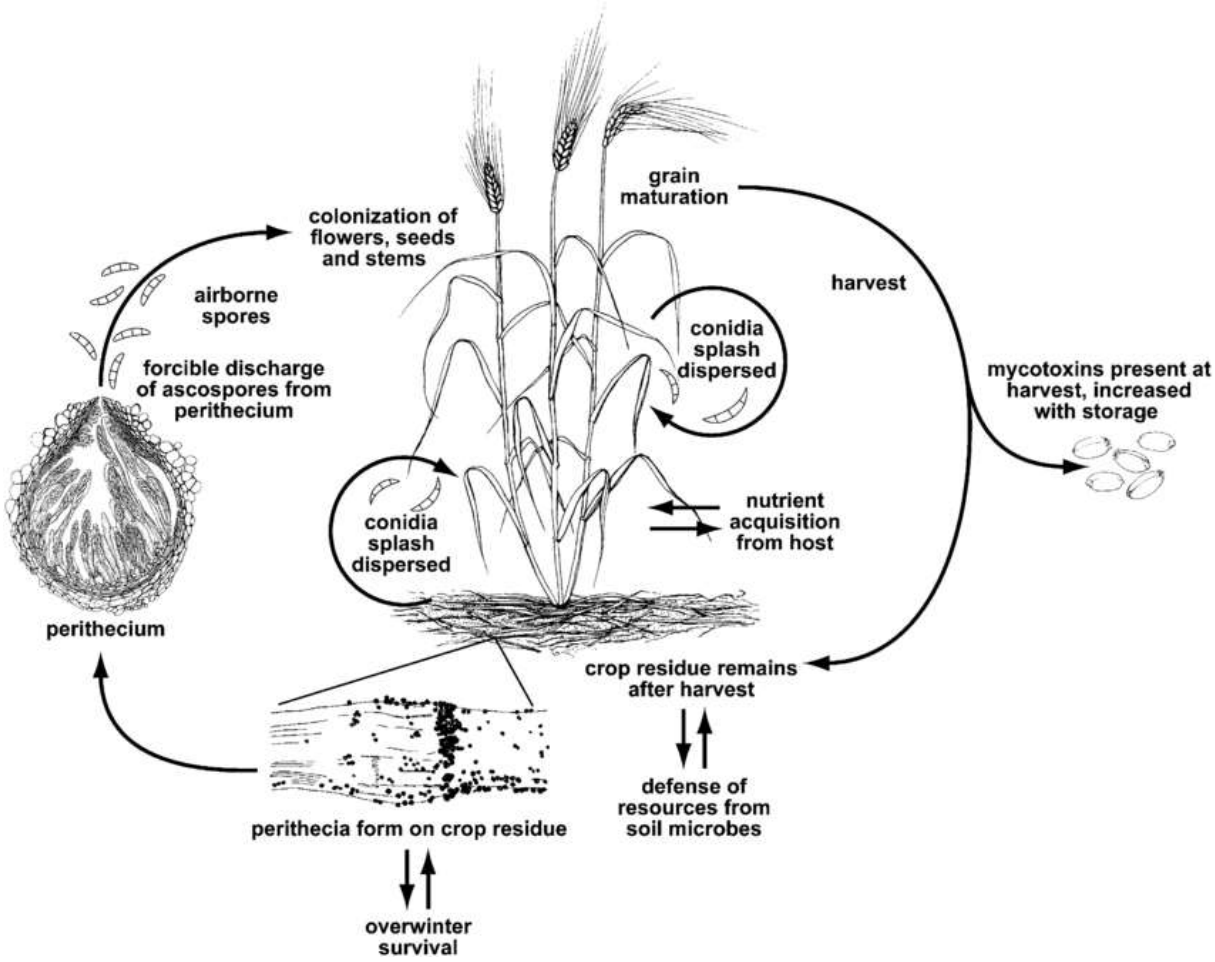

Figure 2.6. An illustration of the disease cycle of Fusarium head blight on wheat (Trail, 2009).

2.5.2. Genetics of FHB resistance

The most effective and reliable method to control FHB is by using host resistance (Yi *et al.*, 2018; Buerstmayr *et al*., 2019). Fusarium head blight resistance is classified as non-pathotype or isolate specific (Mesterházy *et al.*, 2015; Buerstmayr *et al*., 2019). Fusarium head blight resistance are known to be a complex trait under multigenic control which is quantitatively inherited (Yi *et al.*, 2018). The genetic gain in FHB resistance breeding in elite commercial wheat cultivars are slow (Dweba *et al*., 2017a; Figueroa *et al*., 2018). There are five types of FHB resistance mechanisms: Type I is resistance to initial infection, Type II is resistance to spreading of the disease within infected heads, Type III is resistance to DON accumulation (resistance to kernel damage), Type IV is tolerance to FHB or trichothecene toxins, Type V is resistance to accumulation of trichothecene toxins (Yi *et al.*, 2018; Góral *et al.*, 2019). The Type V resistance mechanism is subdivided into class 1 where resistance to accumulation of trichothecene toxins occurs by chemical modification and class 2 is the hindering of trichothecene synthesis (Góral *et al.*, 2019).

Historically, wheat breeding programs focused on Type I and Type II FHB resistance because it was reported to be more environmentally stable (Imathiu *et al.*, 2014). The severity of FHB infections under field conditions are measured by Type I and Type II resistance levels (Góral *et al.*, 2019). Fusarium head blight resistance is more stable and durable when Type I and Type II resistance is incorporated into wheat cultivars (Dweba *et al.*, 2017). Type I resistance is more relevant under high *Fusarium* inoculation and consequent infection pressure. The absence of Type II resistance can result in severe head infection even when the infection pressure is low (Góral *et al.*, 2019).

There is an increase in evaluating other types of resistance to develop cultivars that provides a more adequate control against FHB (Imathiu *et al.*, 2014). Sources of FHB resistance have been identified however it is still a challenge to develop a FHB resistant cultivar (Buerstmayr *et al.*, 2003). The best known highly effective FHB resistance source is the Chinese spring wheat cultivar ‘Sumai 3’ (Buerstmayr *et al.*, 2019). The cultivar carries multiple major and minor QTL’s conferring to FHB

resistance (Zhang *et al.*, 2019). The cultivar 'Sumai3' have been used extensively in global wheat breeding programs since the 1970's (Niwa *et al.*, 2014; Sydenham, 2014). Most of the FHB resistant germplasms originated in regions that frequently experienced epidemics such as China's lower-middle Yangtze River Valley (Ma *et al.*, 2020). Complete immunity or resistance to FHB has not yet been identified in a wheat cultivar (Dweba *et al.*, 2017a). The reasons for this is that resistant germplasms have poor agronomic traits and the resistant trait needs to be adapted in local germplasms (Buerstmayr *et al.*, 2003; Ma *et al.*, 2020).

A relatively large number of quantitative trait loci (QTL's) with small effects controls FHB resistance in spring wheat with no single gene conferring to complete resistance (Dong *et al.*, 2018). The QTL's that are linked to FHB resistance are highly affected by genotype x environment interaction (GxE) (Miedaner *et al.*, 2018). More than 250 QTL's that confers to some level of resistance to FHB have already been described (Tayo *et al*., 2018). Quantitative trait loci associated markers that previously were identified can be used in marker-associated selection when breeding for resistant cultivars (Buerstmayr *et al*., 2008; Dong *et al.*, 2018). Thus far only a handful of these QTL's have been employed successfully in breeding programs (Steiner *et al.*, 2017).

2.5.3. Important QTL's in this study

Quantitative trait loci and genes that confers to FHB resistance have contribute significantly to FHB resistance breeding (Dweba *et al.*, 2017a). Several QTL's conferring to FHB resistance have been mapped on the following chromosomes: 1B, 2D, 3A, 3BS, 4B, 5AS, 5B, 6BS and 7A. Most of the QTL's only have minor effects linked to FHB resistance. A few of the QTL's exert major effects on FHB resistance and have been verified (Xu *et al.*, 2020). Seven major QTL's have been designated with gene names namely *Fhb1, Fhb2, Fhb3, Fhb4, Fhb5, Fhb6* and *Fhb7* (Cai *et al.*, 2019). The QTL's that have been fine-mapped thus far are *Fhb1, Fhb2, Fhb4, Fhb5, Qfhs.ifa-5A, Qfhs.ndsu-3AS, Qfhb.nau-2B and Qfhb.mgb-2A*. Only *Fhb1* and *Qfhb.mgb-2A* have been cloned (Buerstmayr *et al*., 2019).

2.5.3.1. *Qfhs.ndsu-3BS*

Fhb1 (syn. *Qfhs.ndsu-3BS*) was identified 20 years ago as the first major QTL that showed Type II resistance (resistance against spreading of the disease in the spike) to FHB (Buerstmayr *et al*., 2019). The *Fhb1* gene was mapped in the cultivars 'Sumai 3' and 'Ning7840' on chromosome arm 3BS (Kosgey, 2019) The gene encodes for a chimeric lectin that is known to possess biological properties involved in plant defence against pathogens. The discovery of the pore-forming toxin (PFT) gene confers to resistance against Fusarium species resulting in a host resistance (Buerstmayr *et al*., 2019; Rawat *et al.*, 2016). The QTL have been extensively studied and are widely deployed in wheat FHB resistance breeding programs (Buerstmayr *et al*., 2019).

The presence of the QTL might also have DON inhibiting or DON break down mechanisms (Mesterházy *et al.*, 2015). It is suspected that the QTL on chromosome 3BS also encodes for an enzyme that is involve in the production of a DON-glucosyl-transferase (that detoxicates DON in plants) or regulate the expression of it (Niwa *et al.*, 2014; Mesterházy *et al.*, 2015). The presence of *Fhb1* on chromosome 3BS results in higher resistance in plants because it strongly lowers DON production and reduce Fusarium damage kernels (Mesterházy *et al.*, 2015; Buerstmayr *et al*., 2019). The presence of this QTL in wheat plants results in lower infection severity and less contamination of DON (Mesterházy *et al.*, 2015).

2.5.3.2. *Qfhs.ifa-5A*

The major QTL *Ofhs.ifa-5A* (syn. *Fhb5)* was mapped in the cultivars 'Sumai 3', 'Frontana' and 'Wangshuibai' on chromosome arm 5AS (Niwa *et al.*, 2014; Kosgey, 2019). *Qfhs.ifa-5A* was validated in different studies either in combination with other QTL's or individually and it is one of the most frequently studied QTL's (Steiner *et al.*, 2017). A study done by Buerstmayr *et al.* (2003) concluded that the QTL contributes more to Type I resistance rather than Type II. The Type I resistance mechanisms is not well known and therefore has been neglected (Mesterházy *et al.*, 2015). The QTL *Qfhs.ifa-5A* descended from 'Sumai 3', the highly Fusarium resistant cultivar, and contributes primarily to the resistance to fungal entry (Steiner *et al.*, 2017). This QTL's

mechanism is still unclear but is associated with an enhanced lipid transfer protein (Tayo *et al*., 2018).

The chromosomes 5A (*Qfhs.ifa-5A*) and 3B (*Qfhs.ndsu-3BS*) are associated to significantly contribute to FHB resistance (Buerstmayr *et al.*, 2003). These major QTL's are mapped to the same genomic region and contributes to FHB resistance Type I and Type II in a non-specific nature (Buerstmayr *et al.*, 2003; Mesterházy *et al.*, 2015). In a study done by Buerstmayr *et al.* (2003) reported that 40 – 48% of phenotypic variance (depending on the resistance trait) were explained by these two QTL's together. Higher efficiency against FHB can be achieved when these QTL's (5AS and 3BS) are combined in one genetic background. These QTL's are promising for use in marker assisted selection (MAS) breeding programs (Suzuki *et al*., 2012). Both QTL regions are well covered by SSR markers and MAS can be applied to breed and select lines with the combination of Type I and Type II resistance (Buerstmayr *et al.*, 2003). The *Fhb5* region are flanked by the SSR markers *Xgwm304* and *Xbarc117* and the SSR markers *Xgwm493* and *Xgwm533* spans over the *Fhb1* region (Xue *et al.*, 2010; Buerstmayr *et al*., 2019).

2.5.3.3. *Fhb2*

Fhb2 is a major QTL that confers to rachis resistance in the field, and was mapped to the short arm of chromosome 6BS in 'Sumai 3' (Dhokane *et al.*, 2016; Kosgey, 2019). The QTL was derived from a recombinant inbred (RIL) population from a cross between 'AC foremost' (susceptible parent) and 'BW278' (resistant parent) and explained 24.1% of the resistance to FHB in a study done by Dhokane *et al.* (2016). The 'BW278' resistant parent is a cross of the cultivar 'Sumai 3' that also shows high levels of rachis resistance. This QTL region confers to Type II resistance in 'Sumai 3' cultivar (Sydenham, 2014). The QTL's *Fhb2* and *Fhb1* have been associated with activation of the jasmonic acid (JA) pathway, increase defence metabolites from the phenylpropanoid pathway and detoxification of DON to less toxic compounds (Tayo *et al*., 2018). The QTL, designated *Fhb2*, was mapped between the markers *Xgwm644* and *Xgwm133* in a study done by (Cuthbert *et al*., 2007).

2.5.3.4. *Fhb7*

The QTL was introduced into wheat from *Thinopyrum elongatum*, a grass that forms part of the Triticeae family. *Fhb7* shows similar mode of resistance to FHB resistance as *Fhb1.* *Fhb7* confers to a broad resistance against Fusarium species by encoding for a glutathione S-transferase (GST) that can biochemically detox trichothecene mycotoxins via de-epoxidation. This can result in a reduction of DON contamination in wheat. The broad detoxification spectrum of *Fhb7* can detox the mycotoxin DON, produced by multiple *Fusarium* species. The QTL can provide a solution to Fusarium resistance breeding because it confers to resistance of both crown rot and FHB in diverse wheat backgrounds without yield penalty (Wang *et al*., 2020). The QTL was mapped and flanked by the SSR markers *Xcfa2240* and *XsdauK66*. The *Fhb7* marker still needs fine mapping because the marker density of the region was lower than required (Guo *et al.*, 2015). The use of the *Fhb7* gene in wheat breeding is limited because of the lack of the *Thinopyrum* reference genome which hinders the cloning and marker development of *Fhb7* (Wang *et al.*, 2020).

2.5.3.5. *Fhb7AC*

The QTL *Fhb3* (syn. *Fhb7AC*) was introgressed from *Leymus racemosus* and mapped to chromosome arm 7AS (Xue *et al.*, 2010; Kosgey, 2019). The translocation involved the short arm of *L. racemosus* chromosome 7Lr#1 to the long arm of wheat chromosome 7A. This translocation can be used directly in FHB resistance breeding programs (Jayatilake *et al.*, 2011). The QTL *Fhb7AC* that confers to FHB resistance was identified and mapped near the centromere of chromosome 7A in the cultivar ‘Sumai 3’ (Jayatilake *et al*., 2011; Sydenham, 2014). The source of this cultivar was traced back to an Italian cultivar ‘Funo’ which was a parent of ‘Sumai 3’ (Sydenham, 2014).

In the study done by Jayatilake *et al*. (2011) the QTL explained 24% of the phenotypic variation for Type III and 22% associated with resistance to spread in wheat heads. Together *Fhb7AC* and *Fhb1* explained 46% variation for Type III and 56% for Type II. The study also reported 84% reduction in deoxynivalenol (DON) content and

66% reduction in FHB severity. The closest marker to flank *Fhb7AC* is Xwmc17 (Xue *et al.*, 2011).

2.5.3.6. *Qfhi.nau-4B*

The major QTL *Fhb4* (syb. *Qfhi.nau-4B*) was mapped on chromosome 4B in the genotype of 'Wangshuibai'. This QTL contributes to Type I FHB resistance (Xue *et al.*, 2010). The QTL was also associated with another FHB resistance genotype such as 'Wuhan 1' (Kosgey, 2019). In the study done by Xue *et al.* (2010) they reported that the two QTLs *Qfhs.ifa-5A* and *Qfhi.nau-4B* explained 15% of the RIL population's (Wangshuibai germplasm) phenotypic variation. The QTL was flanked by the markers *Xgwm149* and *Xhbg226* and *Fhb4* was designated. Currently the QTL cannot be fine mapped because of the low DNA marker density in that region.

2.5.3.7. *Fhb6*

The major gene *Fhb6* was introgressed from the perennial grass *Elymus tsukushiensis* to wheat and mapped to chromosome 1AS. From a study done by Cainong *et al.* (2015) it was reported that the disease severity was only 7% with the presence of *Fhb6* compared to 37% disease severity with absence of *Fhb6*. *Elymus tsukushiensis* thrives in warm, humid regions of Japan and China and has been identified as a source of Type I and Type II FHB resistance. The major gene *Fhb6* was deployed in 'Everest', the Kansas winter wheat cultivar.

2.5.3.8. *Qfhs.ndsu-3AS, QFhb.nau-2B* and *Qfhb.mgb-2A*

The QTL *Qfhs.ndsu-3AS* was the first FHB resistance report from *Triticum dicoccoides* (wild emmer wheat) providing moderate levels of resistance. The QTL was mapped to chromosome 3A in *T. dicoccoides* genotype. The FHB resistance QTL was identified in a population from a cross between *Triticum carthlicum* and durum wheat (Garvin *et al.*, 2009). The two major QTLs *Qfhi.nau-5A* and *Qfhi.nau-4B* are important sources of Type I resistance found in 'Wangshuibai' resulting in an increase resistance against pathogen infection (Ma *et al.*, 2020).

The QTL *QFhb.nau-2B* was mapped to chromosome 2B and identified in the 'Nanda 2419' wheat cultivar. This QTL contributes to Type I and Type II FHB resistance. It is expected that the QTL originate from lines from 'Akakomugi'. 'Nanda2419' was a selection of 'Mentana', an Italian wheat cultivar, with a pedigree of Rieti/Wilhelmina//Akakomugi (Li *et al.*, 2019a). The Type I resistance is flanked by the markers *Xwgrb1561* and *Xwgrb1410* and Type II is flanked by *Xwgrb1410* and *Xwgrb1503*. Markers that are more closely related to the QTL are needed to be used in breeding programs (Li *et al.*, 2019a).

The QTL *Qfhb.mgb-2A* was mapped in durum wheat on chromosome 2A. The QTL was introgressed from a resistant line derived from 'Sumai 3', the Chinese spring wheat. In the QTL region a wall-associated receptor-like kinase (WAK2) gene was identified to be involved in the FHB resistance mechanism. Screening for FHB resistance in MAS breeding programs can be done by using the SSR marker *WAK2-FHB-2A* (Gadaleta *et al.*, 2019).

2.5.4. Inoculation techniques

The evaluation of FHB resistance are affected by environmental conditions such as temperature and precipitation that are difficult to control (Imathiu *et al.*, 2014). Although molecular markers are available to screen for QTL's conferring to FHB resistance, the most effective approach to identify resistance is by performing reliable phenotyping (Schlang and Duveiller, 2012). To perform FHB phenotyping in wheat, artificial inoculation is essential (Buerstmayr *et al.*, 2003). Artificial inoculation is used to test for the best resistance performance under uniform moderate to high disease pressure (Buerstmayr *et al*., 2019). Indoor inoculation results in more accurate comparisons because it enables more control of the environmental conditions in comparison to outdoor inoculation (Chang *et al.*, 2018).

2.5.4.1. Spray inoculation

Spray inoculation is used to measure the total effect of QTL's which confers to Type I resistance components (Mesterházy *et al.*, 2015). This inoculation technique is the most used to inoculate plants with *F. graminearum* isolates at full anthesis, plant

development stage 10.5 on the Feekes sacle (Figure 2.7) (Schlang and Duveiller, 2012; Góral *et al.*, 2019). Wheat heads should all be inoculated at the same development stage (Góral *et al.*, 2019). The inoculation method involves spraying conidial suspension (spore suspension) onto wheat heads to mimic natural field inoculation. Spore suspensions can be made up of either a mixture of isolates or a single isolate. Suitable plots (field trial) or pots (glasshouse trial) are selected the day before inoculation in order to prepare the necessary amount of inoculum. The spore suspension are stored at 4°C overnight and kept cool during the inoculation process (Sydenham, 2014; Mesterházy *et al.*, 2015).

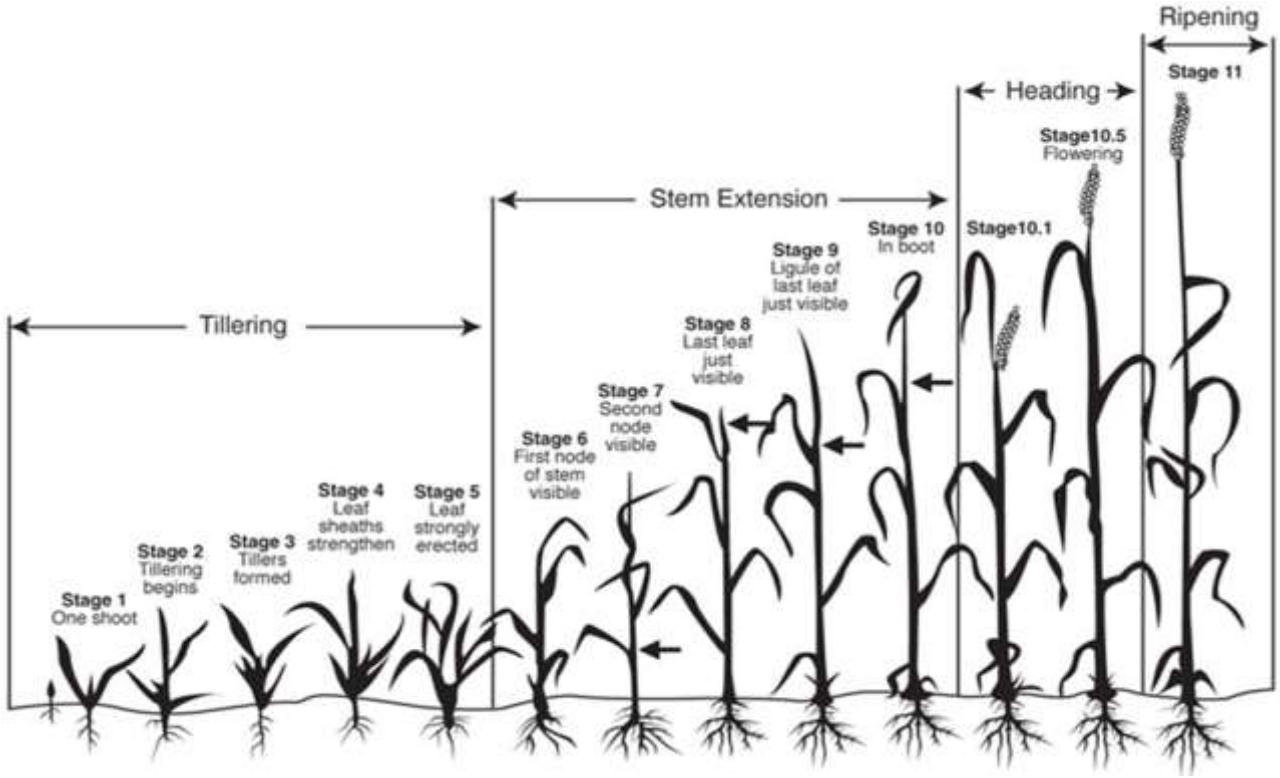

Figure 2.7. The diagram illustrates the growth stages of cereals according to the Feekes scale (Marsalis and Goldberg, 2006).

Mesterházy *et al.* (2015) performed spray inoculation trials in the field and used an amount of 15-20 mL spore suspension that were sprayed on a bunch of wheat heads depending on the sizes of the wheat heads (Mesterházy *et al.*, 2015). Sydenham (2014) performed inoculation trials in the glasshouse by spraying spore

suspension onto wheat heads by using a hand spray bottle. To initiate the epidemic level a certain length of humid period is needed. This could be achieved by irrigation, misting or covering the heads with polyethylene bags for 24-48 hours to create humidity (Mesterházy *et al.*, 2015). The pathogen takes five to seven days after infection to reach the rachis and spread in wheat heads, therefore the number of infected spikelets can be observed a week after inoculation (Góral *et al.*, 2019). The spray inoculation method is simple and highly productive but not very precise. The rating of disease severity can be imprecise because the inoculation time cannot be determined (Mesterházy *et al.*, 2015). It was reported that various environmental factors plays a role in the spray method which makes it unreliable and hard to optimise (Sydenham, 2014).

2.5.4.2. Point inoculation

Point inoculation involves the injection of *Fusarium* spore suspensions into flowers of individual heads (Góral *et al.*, 2019). This inoculation technique mimics the natural infection of cereal florets where the inoculum is transferred by insects such as thrips or aphids which are often found on the crop (Imathiu *et al.*, 2014). The point inoculation method is easier to perform in the glasshouse when evaluating for Type II resistance response (Sydenham, 2014). The development of the disease is assessed by determining the number of flowers that shows symptoms of bleaching or necrosis. The scoring of the disease should occur 21 days after the inoculation. The rate of disease progress can also be determined by scoring several times after inoculation (Góral *et al.*, 2019). Type II resistance assessment are more reliably since the disease symptoms occurs only on inoculated wheat heads (Dweba *et al.*, 2017a). In a study done by Schuster and Ellner (2008) it showed that the number of spores reaching the inside parts of the florets is proportional to the level of Fusarium infections of the wheat ears. The chance of infection is higher when spores are placed inside of the florets. Sydenham (2014) used the cotton wool inoculation method. Cotton balls of 2-3 mm in diameter are dipped in the spore suspension placed within the florets by using tweezers. In a study done by Alisaac *et al.*, (2021) they used a pipette to inject 5 µL of inoculum in between the palea and lemma of the florets. The point inoculation method ensures that an equal amount of inoculum is delivered to plants while also reducing the chance of disease escape. This method also includes bagging of

inoculated heads to create high humidity or using a mist chamber for approximately 48 hours after inoculations to provide favourable disease conditions. Point inoculation is more time-consuming and labour intensive compared to the spray inoculation method (Imathiu *et al.*, 2014).

2.5.5. FHB assessment

Fusarium head blight resistance in wheat are either a result of gene products that contributes to plant defence mechanisms or plant features (such as morphological traits) that have an indirect effect by lowering incidence of disease. Evaluation of reliable FHB resistance is based on well-replicated experiments. The overall field resistance is measured by FHB severity (Type I), FHB spreading (Type II) and FHB severity or area under disease progress curve (Buerstmayr *et al*., 2019). The indirect methods to determine FHB resistance of a genotype is by performing DON analysis and visual scoring (Schlang and Duveiller, 2012). Visual scoring of FHB symptoms is the most common FHB phenotypic screening. The disease incidence and disease severity are determined by estimating or count of the percentage of ears that shows symptoms (Schlang and Duveiller, 2012).

Fusarium damaged kernels (FDK) asses' resistance Type III by determining the proportion of kernels in a grain sample that are damaged throughout infection. The sample of grain are divided into kernels with signs of damage (discoloured-white or shrivelled) and healthy-looking kernels where after the FDK percentage of the sample is calculated (Góral *et al.*, 2019). The FHB index consists of FDK and DON content. Both these measurements can be used singly or in combination when evaluating FHB resistance and are highly correlated (Buerstmayr *et al*., 2019). In a study done by Góral *et al.* (2019) a significant correlation between head infection symptoms and FDK were found, however, there was no correlation between the DON concentration and head infection (visual scoring), as well as FDK.

2.5.5.1. Mycotoxin analysis

A major concern is the accumulation of mycotoxins, especially DON in FHB infected grain (Schlang and Duveiller, 2012). At low concentrations mycotoxins can induce

toxic responses in vertebrates (Agriopoulou *et al.*, 2020). Grain's flour brightness and yield as well as baking performance are also reduced as a result of mycotoxins (Tayo *et al.*, 2018). An important component of FHB phenotyping is the analysis of DON contamination (Schlang and Duveiller, 2012). The FHB disease severity is not always correlated to the DON content of grain. Deoxynivalenol contamination can be detected in grains that appear healthy by visual examination (Nakagawa *et al.*, 2017). Analysing DON contamination are labour intensive and costly (Schlang and Duveiller, 2012). By quantifying the content of *Fusarium* toxins Type IV and V resistance are evaluated in grain. Different methods can be used, such as immunoenzymatic tests, however, chromatographic techniques are more precise (Góral *et al.*, 2019).

2.6. Molecular breeding strategies

Plant breeding is an applied research area that benefits from molecular marker technologies (Ayalew *et al.*, 2019). Plant breeding relies on screening of available genetic diversity, generation of new genetic variation and the selection of superior individuals (Steiner *et al.*, 2017). Selection is an important component in plant breeding. Phenotypic selection is influenced by grow phase of the plant and genotype environment interaction (GxE), therefore the selection method can be inaccurate when selecting a desirable genotype. The development of environment independent methods is more preferred by breeders such as the development of molecular maker technology (Vagndorf *et al.*, 2018). Genomics and molecular markers are supplementing phenotypic selection with genotypic selection in breeding programs (Baenziger and Depauw, 2009). Marker technology is becoming less expensive and more accurate over time (Mundt, 2018). Marker technology has also progressed to handling large population screening which allow the identification of target recombinants at many loci (Bonnett *et al*., 2005). By using molecular breeding strategies selection can be performed earlier before cost-intensive phenotyping. This will result in an increase of gain by selection per unit time and the potential shortening of a breeding cycle (Steiner *et al.*, 2017).

The marker-based strategy known as genomic selection (GS) make use of available molecular marker information that covers the whole genome to predict progenies genetic value for selection (Gokidi *et al*., 2016). Genomic selection serves

as a complementary/alternative method of genotypic selection which supports the breeding of complex quantitative traits such as breeding for FHB resistance. Genomic selection can exploit all the QTLs present in the primary gene pool. It can be used to predict the estimated genomic breeding values for individuals in a population not phenotyped for the trait (Steiner *et al.*, 2017). When GS is validated it can be implemented in MAS which will result in improvement of efficient and accurate selection of quality parameters, disease resistance and multi-genic traits (Vagndorf *et al.*, 2018).

2.6.1. Marker assisted selection

Marker assisted selection is the indirect selection of a genotype based on it carrying desirable genes that could be detected through genetic markers. Marker assisted selection helps to monitor the absence or presence of chromosomal segments that contains the desired genes in breeding populations (Gokidi *et al*., 2016). This breeding method also helps to identify genes that is involved in the expression of complex traits such as the detection of QTLs (Lado *et al.*, 2017). This method is independent and more reliable than phenotypic validation (Wessels and Botes, 2014).

By using MAS, the evaluation of genes can be done in a shorter time (increased efficiency) on a larger scale which will also decrease costs (Baenziger and Depauw, 2009; Wessels and Botes, 2014). Marker assisted selection has become a standard procedure in breeding programs (Vagndorf *et al.*, 2018). Marker assisted selection in wheat breeding makes use of several types of markers which includes RFLP, AFLP, SSR, SNP and diversity arrays technology (DArT) markers (Nadeem *et al.*, 2018). Marker data would still be needed to be linked with reliable and precise phenotypic evaluations (Baenziger and Depauw, 2009).

Genome-scale genotyping platforms are less flexible and expensive to use as a routine genotyping tool. Therefore, the utilizing of small-scale SNP genotyping are becoming more preferred among breeders (Ayalew *et al.*, 2019). Marker assisted selection has been restricted by lack of efficient molecular markers that ineffectively mapped genes in a low gene density chromosomal region (Wu *et al.*, 2017). In current

plant breeding programs small-scale high throughput genotyping systems are in high demand.

Competitive allele specific PCR (KASP) is a small-scale high-throughput SNP genotyping platform. KASP collects data after the PCR process is completed (post-read technology) and was developed to improve genotyping efficiency and reduce costs (Ayalew *et al.*, 2019). KASP was initially developed by KBioscience and is a homogenous, fluorescence-based genotyping technology that evolved onto a global benchmark technology. It is based on fluorescence resonance energy transfer (FRET) and allele-specific oligo extension (Semagn *et al.*, 2014). The disadvantage of KASP is that it has a low allele cluster separation and requires a higher amount of DNA template (Ayalew *et al.*, 2019). KASP has become rapidly adopted in MAS and mapping studies (Wu *et al.*, 2017).

KASP has a potential application to be applied in breeding programs. KASP is useful when phenotyping is expensive and laborious to apply in breeding programs (Semagn *et al.*, 2014). KASP has become the chosen marker system in wheat because it is efficient, locus specific and low cost (Wu *et al.*, 2017). KASP is a breeder friendly fluorescence-based, high-throughput genotyping platforms for SNP markers (Singh *et al.*, 2019). International maize and wheat improvement center (CIMMYT) have been using the KASP platform routinely to systematic mine through large germplasms collections to identify accessions with desirable alleles at target loci (Semagn *et al.*, 2014). KASP markers in wheat has been developed for stem rust resistance, leaf rust resistance, pre-harvest sprouting resistance, wheat streak mosaic virus resistance and for FHB resistance gene *Fhb1* (Singh *et al.*, 2019). It was reported by Qureshi *et al.* (2018) that KASP markers *sunKASP_224* and *sunKASP_225* can be used to detect the presence of the *Sr26* gene and apply it to marker assisted pyramiding. In a study Singh *et al.* (2019) reported the PFT_KASP marker for detecting *Fhb1* and can be a valuable tool used in pyramiding FHB resistance in wheat cultivars.

2.6.2. Marker assisted backcrossing

Backcrossing are described as a recurrent hybridization method where an undesirable or alternative allele is substituted for a desirable allele correlated to trait of interest

(Baenziger and Depauw, 2009). Backcrossing method is used to increase the frequency of recurrent parent alleles (Bonnett *et al*., 2005). The effectiveness of the backcross method depends on the following: (i) heritability of the trait, (ii) the expression of the trait and its phenotypically marker detection, (iii) the degree of independent expression of the trait in the background of other genes, (iv) the linkage of undesirable genes with desirable genes, and (v) the number of backcrosses needed to recover a desirable level phenotype of a recurrent parent (Baenziger and Depauw, 2009). It is a common breeding method to introgress genes or traits of interest into elite germplasm by transfer genes from wild relatives into breeding material of modern cultivars (Ragot *et al*., 1995; Vagndorf *et al.*, 2018). The advantage of backcrossing is that it does not disrupt the genetic balance of targeted modifications in the recurrent parent (Ragot *et al*., 1995).

In theory an average of seven backcrossing generations are needed to recover more than 99% of the recurrent parent's genotype when no linkage drag is assumed. The classical backcrossing procedure is seen as time consuming. The use of molecular markers in a marker assisted backcrossing (MABC) procedure can reduce the number of backcrossing generations needed (Ragot *et al*., 1995). It would also easily detect desired genes in offspring and reduce linkage drag (Vagndorf *et al*., 2018). When the translocation is high backcross may lead to introduction of chromosomes or fragment of chromosomes in progenies (Bouguennec *et al.*, 2018). The MABC method will provide important quality and time advantages compared to the classical backcross procedure (Ragot *et al*., 1995). A study was done by Ragot *et al*. (1995) that reported with marker assisted backcrossing only four backcrossing generations were needed. Marker assisted backcrossing breeding strategy has been successfully employed in wheat programs (Yadav *et al.*, 2015).

2.6.3. Recurrent mass selection

Recurrent mass selection (RAS) involves the selection, evaluation and recombination generation after generation. Repeating inter-mating between selected plants occurs to produce the next cycle of selection of a heterozygous population (Gokidi *et al*., 2016). The aim of RMS is to maintain the genetic diversity in a base population while

increasing the frequency of desirable genes (Marais and Botes, 2009). Recurrent mass selection can either be used for general combining ability (GCA) or specific combining ability (SCA). The breeding method is considered as an effective strategy to improve polygenic traits while maintaining high genetic variability (Gokidi *et al*., 2016). Recurrent mass selection is a very popular method used to increase the frequency of desirable alleles to improve a breeding population (Marais and Botes, 2009; Gokidi *et al*., 2016). The breeding technique is well established in cross-pollinated crops for genetic improvement. The high level of inherited heterozygosity combined with large number of cross combinations results in higher polygenetic recombination potential in breeding populations (Marais and Botes, 2009). In cross pollinated crops it leads to forming of new gene combinations by breaking up existing linkage blocks (Marais *et al*., 2001). The implementing of RMS has shown to be successful when improving of polygenic traits in cross-pollination crops. The application of RMS in self-pollinating plants (such as small grain cereals) were restricted to the amount of intercrosses that could be made. It was suggested that RMS be integrated with other selection methods when breeding with self-pollinated crops (Marais and Botes, 2009).

2.6.4. Marker assisted recurrent selection

The marker assisted recurrent selection (MARS) scheme involves the identification and crossing of selected individuals for several generations based on molecular marker genotypes. Genotypic selection and intercrossing can occur in the same crop cycle when applying the MARS scheme. This scheme makes use of molecular markers to identify and select multiple genomic regions that is associated with the expression of complex traits to end up with the best performing genotype. The MARS approach is used when dealing with the expression of complex traits where multiple genes or QTL's plays a role. Multiple desired genes and QTL's from different sources can be integrated by recurrent selection based on multi-parent populations. This breeding scheme can result in high genetic gain. The MARS scheme has been proposed for complex traits such as abiotic and biotic resistance and grain yield as a

breeding method of native genes as well as pyramiding multiple QTL's (Gokidi *et al*., 2016).

2.6.5. Male sterility marker assisted recurrent selection

Self-pollinating crops can rapidly fix genes, because heterozygosity is halved after every successive generation resulting in reducing the opportunity for genetic recombination. The problem with implementing RMS into breeding of self-pollinating crops was resolved by using a male sterility gene. The male sterility gene helps to facilitate mass crossings in wheat lines to breed for multi-genic pest resistance (Marais and Botes, 2009). The male sterility trait is a valuable tool for plant breeding and hybrid seed production (Ni *et al*., 2017). Male sterility genes enhance outcrossing of natural self-pollinating crop species such as wheat.

Genetic male sterile genes are mutants that are produce by ethyl methane sulphonate (EMS) seed treatment (Maan and Kianian, 2001). Genetic male sterility (GMS) causes abnormal stamens, due to stamen degeneration, and abortion of pollen. Five GMS genes have been located on hexaploid wheat chromosomes. The genes *Ms1* and *Ms5* are recessive genes that are located on chromosome 4B and 3AL. The other three male sterility genes known as *Ms2, Ms3* and *Ms4* are dominantly inherited and are located on chromosome 4DS, 5AS and 4DS (Li *et al.*, 2019). The *Ms2* gene was mostly used to develop varieties with improved quality, adaptation and scab resistance (Marais *et al*., 2001). The gene confers to genetic male sterility in tetraploid and hexaploid wheat. Regardless of environmental conditions, phytohormones and genetic background the *Ms2* gene confers to 100% male sterility. Wheat breeding programs in China uses the *Ms2*-based recurrent selection systems to facilitate breeding of cultivars and lines (Ni *et al.*, 2017).

When the *Ms3* gene is used in a recurrent selection breeding population segregation of male fertile and male sterile (female) plants occurs (Marais and Botes, 2009). The gene results in enforcing cross-pollination of selected plants that are naturally self-pollinating crops (Marais *et al*., 2001). The gene has a stable expression in glasshouse conditions that range between 16 and 25°C. Incomplete penetrance of this gene occurs when temperatures range between 21 and 35°C (summer glasshouse

condition). The *Ms3* gene helps to facilitate recurrent selection in wheat based on open pollination (Marais *et al*., 2001). In this breeding strategy the male fertile plants were pollinated by selected male sterile (female) plants (Marais and Botes, 2009). Marais *et al*. (2001) have reported that utilizing the dominant male sterile in recurrent selection wheat breeding is feasible

Marais *et al*., (2001) established a recurrent selection base population at Stellenbosch University by utilizing the *Ms3* dominant male sterility gene. This system utilizes random intercrossing of selected parents on large scale in the glasshouse (Marais and Botes, 2009) (Figure 2.8.). The *Ms3* gene has low recombination which complicates positional cloning (Ni *et al.*, 2017). In a study done by Marais *et al*. (2001) the *Ms3* gene was used to establish a recurrent selection base population that segregates into a 1:1 male sterile and male fertile plants.

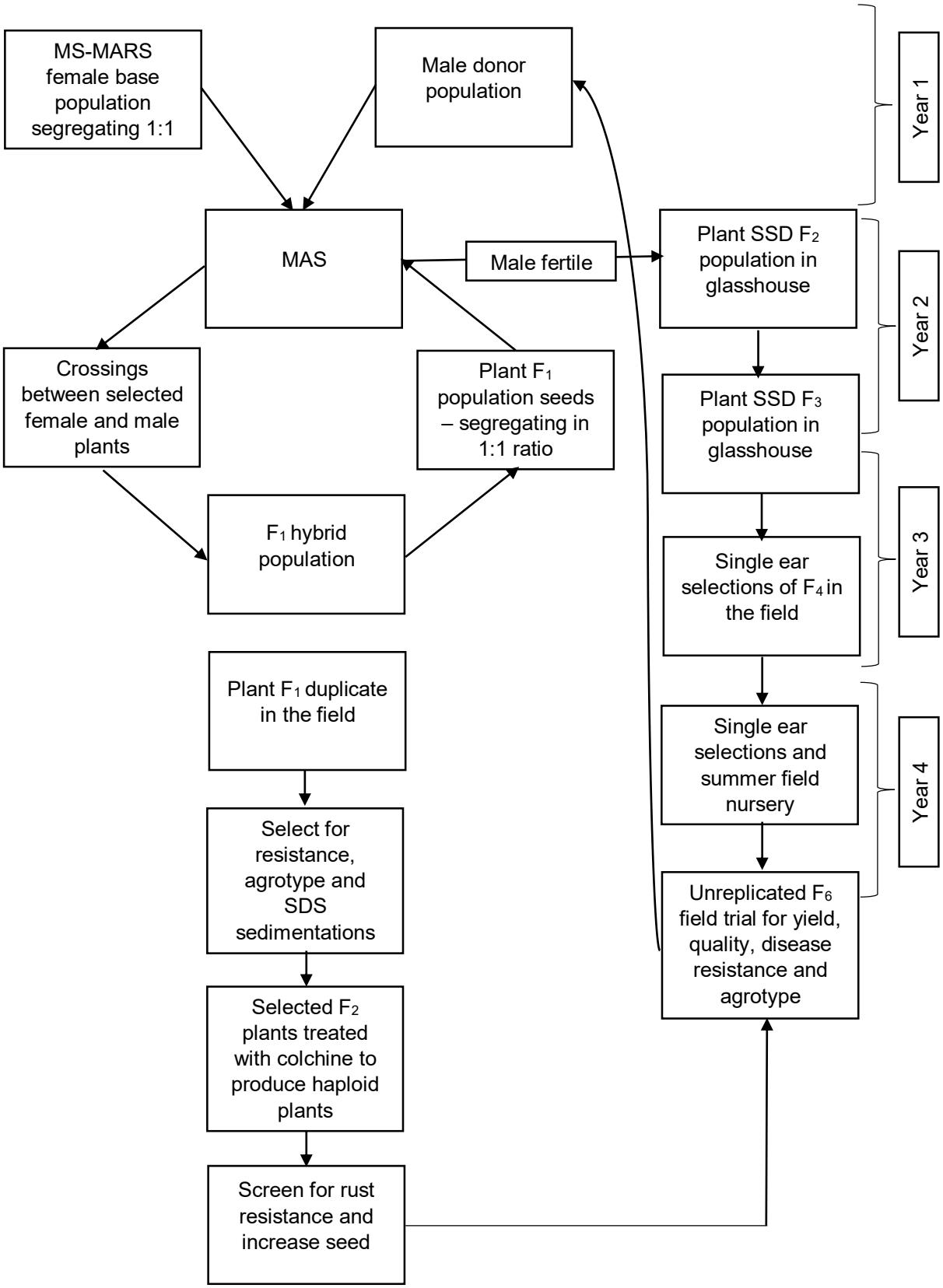

Figure 2.8. Illustrating the male sterility marker assisted recurrent selection breeding scheme (Adapted from Marais and Botes, 2009).

The MS-MARS facilitates a wheat breeding program strategy involves a four-year breeding cycle and selection in initial stages of a highly diverse base population. Selection of simple and highly heritable traits takes place in the initial stages. This breeding cycle can be extended to improve the selection gain of quantitative traits with low heritability. When the base population reached the F_6 stage male selection is done based on its performance at a single locality in a nonreplicated single row. The F_6 rows are used to evaluate yield, disease resistance, agrotype and quality (Marais and Botes, 2009). The presence of *Ms3* gene results in lower seed set on male sterile ears. To promote cross-pollination and higher seed set, florets are cut open at the same flowering time to facilitate intercrossings (Marais *et al*., 2001; Marais and Botes, 2009). Pollination of selected fertile spikes with large numbers of male sterile spikes are facilitated by the development of a hydroponic system (Marais *et al*., 2001). During pollination the tillers are kept in a hydroponic system until seed ripens in the female tillers.

2.7. Stacking or pyramiding of genes

To achieve agricultural sustainability and attaining global food security it is important that disease resistance of cultivars is durable (Mundt, 2018). Pyramiding of resistance genes are described as combining different resistance genes into one breeding line or cultivar which will result in an increase of the resistance longevity against a pathogen. This is a useful strategy to create more broad-spectrum and durable resistances (Zhang *et al.*, 2019).

By using conventional breeding to pyramid major resistance genes into a single genotype can be laborious and time consuming (Yadawad *et al.*, 2017). The pyramiding of durable resistance genes into an elite background can be facilitated in a cost-effective manner and in less time by using the available molecular markers in a MAS scheme (Das *et al*., 2006; Zhang *et al.*, 2019). Molecular marker implimentation has proven to be a useful technique for pyramiding desirable genes with resistance into one background (Yadawad *et al.*, 2017). This technique has been utilized in successfully pyramiding of leaf rust resistance genes (Yadawad *et al.*, 2017). An ideal strategy will be pyramiding different disease resistance genes such as rust and FHB

resistance. This will reduce the loss of crop yield caused by multiple pathogens (Zhang *et al.*, 2019).

Chapter 3: Materials and methods

3.1 Introduction

DNA extractions were performed on the 21st Fusarium head blight (21FHBSN_015) nursery from CIMMYT (Mexico) and the Stellenbosch University's 2018 18US1M (F_{6}-generation) male fertile population obtained from the SU-PBL's MS-MARS pre-breeding program. The nursery and population were characterised by screening with the SU-PBL's standard set of molecular markers that are routinely used. The standard panel of markers were developed for molecular screening of rust resistance, baking quality and yield determining traits.

In the MS-MARS cycle 1 cross-pollination between selected wheat lines (based on molecular data) from SU-PBL's 2018 F_6-population and the segregating male sterile population occurred. Due to the male sterility the population segregates into a ratio of 1:1 male sterile and male fertile plants. The male sterile population obtained the rust resistance genes *Sr2* and *Lr34* through multiple RMS cycles and formed the base population for this study. The F_1 seeds obtained from the female plants in MS-MARS cycle 1 formed the new segregating male sterile population. The male sterile population was molecularly screened for gene combinations utilizing MAS. The new segregating male sterile population was cross pollinated with selected lines from the 21FHBSN_015 nursery and F_6-population that acted as pollen donors in the MS-MARS cycle 2.

Selected wheat lines from SU-PBL's 2018 F_6-population were involved in a leaf and stem rust phenotyping in the field. The wheat plants were inoculated with virulent rust pathotypes. The phenotyping entailed scoring the disease severity with different percentage intervals. Fusarium head blight phenotyping as also performed on selected lines from the 2018 F_6-population and the 21FHBSN_015 nursery over two seasons in the glasshouse. The wheat lines were inoculated with DON-producing *F. graminearum* isolates using both the spray and point inoculation methods. The phenotyping entailed measuring the FHB disease incidence and severity at three time periods after inoculation and also performing mycotoxin analysis of the resultant grain.

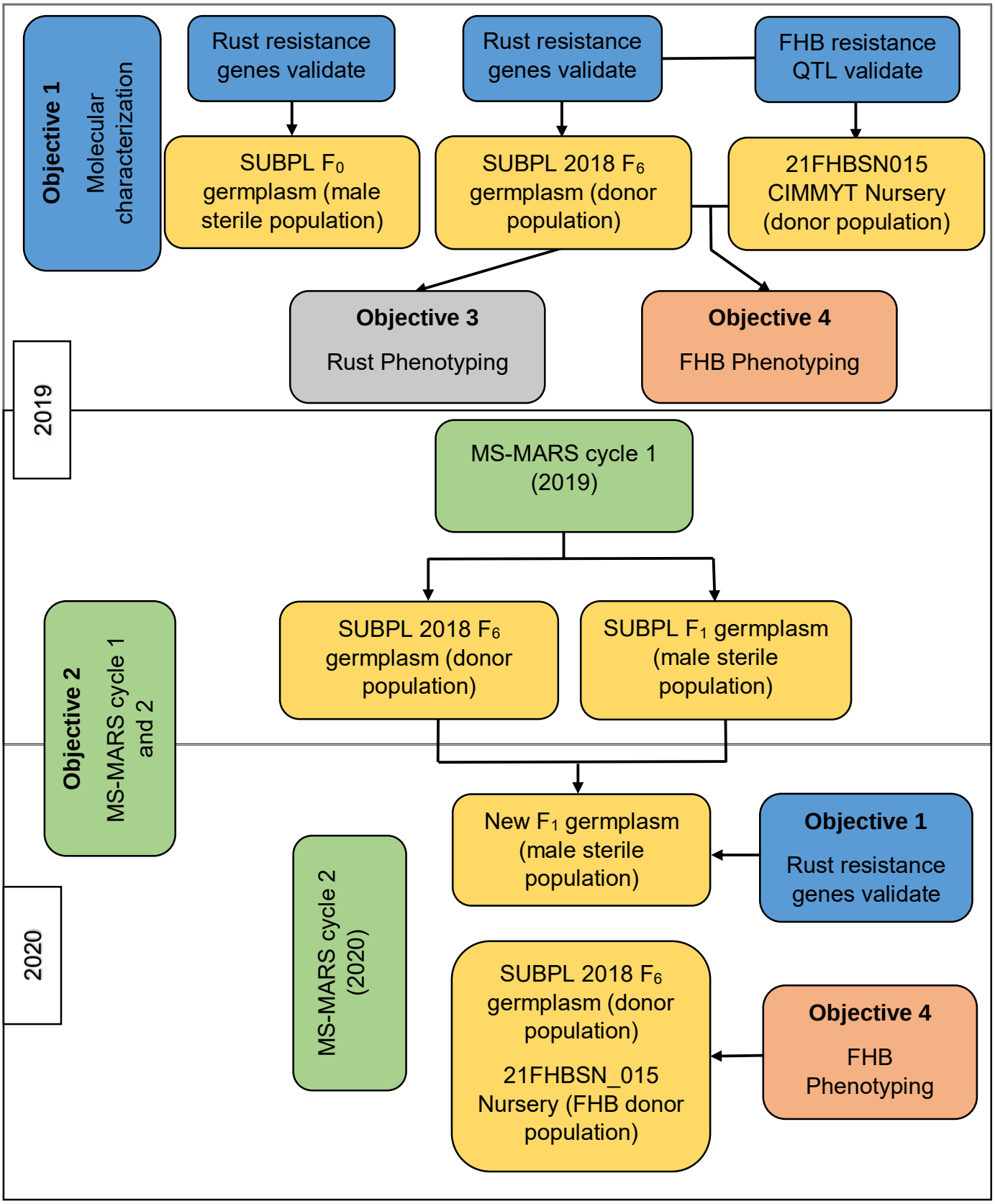

Figure 3.1. An illustration of the work flow of this study.

3.2. Planting

For DNA extraction, planting of seeds were done in growth trays in a growth chamber following a natural light-dark photoperiod at temperatures that ranged between 16-25°C at Welgevallen Experimental Station (WES), Stellenbosch. Three seeds of every wheat line were planted in a potting soil mix. The seedlings received water once a day.

The planting of the male and female population seeds for the MS-MARS cycles occurred in the same glasshouse with four seeds planted per line in black plant bags (2.0 L - 125 x 105 x 230 mm) filled with a coarse sand mix. Planting of the FHB phenotyping material occurred in (9 L – 175 x 150 x 350 mm) black plant bags with 4-9 seeds per line. The plants in the glasshouses were irrigated with a nutrient solution of 2g Microplex (Ocean Agriculture Pty Ltd, Muldersdrift, South Africa), 164g Sol-u-fert T3T (Kynoch Fertilizers Pty Ltd, Milnerton, South Africa), 77 mL potassium nitrate in 100 l H2O) and 0.05% Jik (household detergent containing 3.5% sodium hypochlorite, Reckitt and Colman South Africa Pty Ltd., Elandsfontein, South Africa) daily.

Table 3.1. Wheat germplasm used in this study.

Germplasm/ nursery	Date of planting	Number of lines	Location	Experiment	Year
SUPBL's 2018 F_6-population	22, 23 and 24 January	624	Growth Chamber 3 (WES)	Molecular screening	2019
	April	250	Makhathini Research Station, KwaZulu-Natal	Rust phenotyping (leaf and stem rust)	2019
	9 and 23 July, 6 and 20 August	30	Glasshouse 1 (WES)	Pollen donors for MS-MARS cycle 1	2019
	17 July	29	Glasshouse 4 (WES)	FHB phenotyping on selected lines	2019
	11 June	13	Glasshouse 4 (WES)	FHB phenotyping on selected lines	2020
	16 and 30 June, 14 and 28 July	13	Glasshouse 1 (WES)	Pollen donors for MS-MARS cycle 2	2020
2018 male segregating population	2, 16, 23 July and 13 August	420	Glasshouse 1 (WES)	Molecular screening of female plants for MS-MARS cycle 1	2019

Germplasm/ nursery	Date of planting	Number of lines	Location	Experiment	Year
21FHBSN_015 CIMMYT nursery	17 July	27	Glasshouse 4 (WES)	FHB phenotyping	2019
	15 July	27	Growth chamber (WES)	Molecular screening	2020
	11 June	13	Glasshouse 4 (WES)	FHB phenotyping on selected lines	2020
	16 and 30 June, 14 and 28 July	13	Glasshouse 1 (WES)	Pollen donors for MS-MARS cycle 2	2020
F_1 produced seeds from MS-MARS cycle 1	9 and 23 June, 7 and 21 July	420	Glasshouse 1 (WES)	Male sterile (female) plants used in MS-MARS cycle 2	2020

3.2.1. DNA extraction of plant material

Two weeks after the seeds were planted, leaf pieces (2 cm) of each plant sample were placed into microcentrifuge tubes. An adapted DNA extraction protocol of Doyle and Doyle (1987) was followed. Three stainless steel balls of 3 mm sizes and 500 µL of 2% (w/v) cetyltrimethylammoniumbromide buffer (CTAB) [100 mM Tris-Cl (pH 8.0) 1.4 M NaCl, 20 mM Ethylenediamine tetra acetic acid disodium salt dehydrate (EDTA) (pH 8.0)] was added to each tube. The tubes were then placed in the high-speed Qiagen® Tissue Lyser (Qiagen, Southern Cross Biotech, Claremont, RSA) at 30 Hz for 40 seconds. The tubes were incubated in a water bath at 55-60°C for 20 minutes. After incubation 500 µL of chloroform:isoamylalcohol (24:1) was added to each tube whereafter the samples were centrifuged at 14 000 rpm for 5 minutes. The supernatant of each sample was transferred to a new 1.5 mL microcentrifuge tube. Five hundred

microliters of chloroform:isoamylalcohol (24:1) was added and mixed by inversion. The samples were centrifuged for 5 minutes at 14 000 rpm. The supernatant was then again transferred to a new 1.5 mL microcentrifuge tube. Additionally, 50 µL of 3 M Sodium Acetate (pH 5.5) and 500 µL of ice cold 100% ethanol were added to each tube. The samples were then slowly inverted several times and centrifuged at 14 000 rpm for 5 minutes. After centrifuging the supernatant was carefully discarded and 1 mL of 70% ethanol was added and the samples centrifuged for 3 minutes at 14 000 rpm to wash the pellet. The washing step was repeated three times after which the pellet was dried in the oven for 10 minutes at 60°C. The concentrated and purified pellet was subsequently dissolved in 6-20 µL distilled water (dH_2O) and stored at -20°C.

The genomic DNA concentration from the plant samples was measured by using a Nanodrop® ND-1000 spectrophotometer (Thermo Fisher Scientific Inc., Kempton Park, RSA). Dilutions were made to a final concentration of 100 ng/µL using dH_2O and stored at 4°C to -20°C. The amount of DNA that was added the dilutions was calculated from the results obtained by the Nanodrop.

3.3. Genotyping of plant material

The primers were used in this study was obtained from Integrated DNA Technologies (Whitehead Scientific Inc, Stikland, RSA) and had an initial concentration 10 µM. The KAPA Green Readymix obtained by KapaBiosystems (distributed by Roche (Pty) Ltd, Cape Town, RSA), One Taq Quick-Load 2x Master Mix with Standard buffer from New England BioLabs (distributed by Inqaba Biotechnical Industries (Pty) Ltd) and Ampliqon Red Readymix (Lasec SA (Pty) Ltd, Cape Town, RSA) was used in PCR (polymerase chain reaction) reactions. All PCR reactions were performed by using a 2720 Thermal Cycler (Applied Biosystems, Fairlands, RSA) or a TECHNE TC-5000 (Lasec, Cape Town, RSA).

The 642 wheat lines of the SUPBL's 2018 F_6 germplasm were firstly screened for the presence of rust resistance genes *Sr2, Lr34, Sr24, Lr37* and *Lr19*. A total of 250 lines were selected based on molecular data generated. After the selection the

population was further screened for the presence of the stem rust resistance genes *Sr26* and *Sr31*. A list of these markers are showed in Table 3.2.

Based on the molecular data generated, 30 lines were further selected and screened for the baking quality marker *GluxDx* and yield markers *TaGS-D1-7D*, *Ppd-D1-2D*, *TaGW2* and *TaGS5.* The yield markers are linked to the following wheat characteristics: grain weight, width, size and photoperiod insensitivity (Rhoda, 2018). The selected lines were also screened for *Qfhs.ifa-5A, Qfhs.ndsu-3BS* and *7AQTL* that are linked to FHB resistance. These markers form part of a standard panel of markers routinely used by the SUBPL and the conditions of each marker's PCR reaction is displayed in Table 3.2. The separated PCR products were visualized on agarose gels and polyacrylamide gel- electrophoresis (PAGE) gels.

The 21FHBSN_015 nursery from CIMMYT was also genotyped for the rust resistance, baking quality, yield and FHB resistance markers. The 2018 F_1 male segregating population and the F_1 produced seeds from MS-MARS cycle 1 were only screened for the presence of the stem rust resistance gene *Sr2* and the leaf rust resistance gene *Lr34.*

3.3.1. Screening for rust resistance genes

The PCR reaction of the *Sr2* marker had a final volume of 12.9 µL which contained 7.5 µL KAPA Green Readymix, 3 µL of water, 0.45 µL of *CSSr2* forward and reverse primer and 1.5 µL DNA. The PCR reaction conditions were as follow: denaturation for 2 minutes at 95 °C, 40 cycles of 30 seconds at 95 °C, 60°C for 40 seconds and 72°C for 50 seconds and a final extension of 5 minutes at 72°C. The PCR products were visualized on 1% (w/v) agarose gels stained with 4% (v/v) of Ethidium bromide (EtBr) by loading 5 µL of each sample on the gel. The gels were run at 120 V within 1X TBE [5X TBE stock solution: 0.5 M Tris (hydroxymetyl) Aminomethane, 0.5 m Boric acid, 0.5 M Ethylene-diamine-tetraacetic acid (EDTA)] buffer. For the genotyping, the cultivar 'Steenbras' served as positive control, while 'Chinese Spring' and dH_2O served as negative controls. Positive amplification of the marker at a band size of 337 bp was digested with *BspHI* restriction enzyme. A final volume of 2.5 µL enzyme mix that consisted out of 1X Buffer, 1U/µl of *PagI* enzyme (Thermo Scientific) and brought to

volume with nuclease-free water was added to the PCR products that was left over. The enzyme digestion was incubated for 20 minutes at 37°C and visualized on 2% (w/v) agarose gels.

The PCR of the multiplex containing the *Lr34, Lr19, Sr24* and *Lr37* had a final volume of 18.4 µL. The PCR reaction mix consisted out of 12 µL KAPA Green Readymix, 0.5 µL *SCS719* forward and reverse primers, 0.5 µL *LN2* forward primer and *VENT* reverse primer, 0.85 µL 12C forward and reverse primers, 0.6 µL *Dint9* forward primer and *L34+* reverse primer and 1.5 µL DNA. The PCR conditions of the reaction were as follow: denaturation at 94°C at 5 minutes, 35 cycles of 1 minutes at 94°C, 57°C for 1 minute, 72°C for 1 minute and a final extension for 7 minutes at 72°C. The amplified PCR product was visualized on 1.8 % (w/v) agarose gels. The cultivar 'Chinese Spring' showed positive amplification at 571 bp for the *Lr34* gene. The genotype 'W84-17' was used as positive control for *Sr24, Lr37* and *Lr19* genes that showed amplification at 719 bp, 259 bp and 119 bp. Water was used as a negative control.

The population was also screened for the *Sr26* and *Sr31* rust resistance genes. The multiplex PCR reaction had a final volume of 16.80 µL. The reaction mix consisted out of 1.00 µL *iag-95* (*Sr26* gene) forward and reverse primer, 0.60 µL *Sr31#43* (*Sr31* gene) forward and reverse primer, 2.00 µL Mg_2Cl_2 (25mM), 3.10 µL water and 8.50 µL KAPA Green Readymix. The PCR reaction consisted out of a denaturation of 94°C for 5 minutes, 35 cycles of 94°C for 30 seconds, 30 seconds at 55°C and 72°C for 1 minute and a final extension of 72°C for 7 minutes. The amplified PCR product was visualized on 1.8% (w/v) agarose gel. Positive amplification for *Sr26* produced a 207 bp band a 1000 bp amplification band for *Sr31*. The cultivars 'Eagle' and 'Gamtoos' were used as positive controls for *Sr31* and *Sr26,* respectively water was used as negative control.

3.3.2. Screening for baking quality marker

The population was screened for the *GluxDx* gene which amplified the Dx5, Dy10 and Dy12 alleles. The PCR reaction consisted of the following: an initial denaturation of 94°C for 5 minutes, 94°C for 30 seconds, 63°C for 30 seconds, 72°C for 30 seconds with a duration of 30 cycles and a final extension of 72°C for 5 minutes. The PCR

reaction had a final volume of 18.50 µL and consisted out of 0.75 µL *P1, P2, P3* and *P4* primers, 3.50 µL water and 12.50 µL KAPA Green Readymix. The PCR amplifications were visualized on a 2% (w/v) agarose gel. The cultivar 'Pavon' was used as a positive control with amplification products of Dx5 at 450 bp and Dy10 at 576 bp. The cultivar 'Chinese Spring' was used as a negative control and showed positive amplification of Dy12 at 612 bp while water served as a no template control.

3.3.3. Screening for markers associated with yield

The gene, *TaGS-D1-7D,* that is associated with the thousand kernel weight characteristic was also screened for. Positive amplification of this gene includes two alleles namely *TaGS-D1a* and *TaGS-D1b*. The PCR reaction mix contains 5 µL of Red Readymix, 2.5 µL water, 0.25 µL *GS7D* forward and reverse primer and 2 µL of DNA. The PCR reaction conditions is as followed: the denaturation was at 94°C for 30 seconds, a duration of 30 cycles at 94°C for 30 seconds, 52°C for 50 seconds, 68°C for 60 seconds and a final extension for 5 minutes at 68°C. The PCR products were run on a 3% (w/v) agarose gels. The amplicons showed a band size of 562 bp (*TaGS-D1a*) and 522 bp (*TaGS-D1b*). The positive controls that were used for this marker was the cultivars 'Chinese Spring', 'Pavon' and 'Opata' that showed amplification at 562 bp. Water was used as the negative control for this marker.

The population was screened for the *Ppd-D1-2D* marker that is associated with the grain filling trait. The marker's PCR reaction had a final volume of 14.00 µL which contained 0.50 µL *Ppd-D1-1* forward primer, *Ppd-D1-1* and *Ppd-D1-2* reverse primers, 8.50 µL Red Readymix and 4.00 µL water. The separation of the PCR products was visualized on 2% (w/v) agarose gels. The marker consisted out of two alleles that showed a band pattern at 288 bp and 414 bp. The cultivars were 'Inia66' and 'W84-17' was used as positive controls for the 288 bp allele. The positive control for the 414 bp allele was the cultivars 'Eagle' and 'Chinese Spring'. The PCR reaction consisted out of the following: an initial denaturation for 30 seconds at 94°C, a duration of 44 cycles for 30 seconds at 94°C, 54°C for 30 seconds, 72°C for 30 seconds and a final extension for 10 minutes at 72°C.

The population was screened with the markers *TaGW2* and *TaGS5* that are linked to the following characteristics: a thousand kernel weight, grain width and larger kernel size. Both markers has the same PCR conditions: denaturation for 30 seconds at 94°C, a duration for 13 cycles at 94°C for 30 seconds, 62°C for 1 minute and 68°C for 1 minute, a duration of 17 cycles for 30 seconds at 94°C, 50 seconds at 55°C, 1 minute for 68°C and a final extension of 5 minutes at 68°C. The PCR reaction mix for both markers was also the same and contained 5 µL Red Readymix, 0.25 µL *TaGW2-6B-CAPS* forward and reverse primer, 2.5 µL water and 2 µL of DNA. For both the markers 4 µL of the PCR products were run on 1% (w/v) agarose gels. The PCR products of both markers, *TaGW2-6B TaGS5-3A*, were treated with the *BSTNI* and *Fnu4HI* digestion enzymes. Then after the incubation the PCR products were run on 2% (w/v) agarose gels.

The enzyme *BSTNI* was added to the leftover PCR product of the *TaGW2-6B* marker. The enzyme mix was made up of 4.3 µL water, 0.5 µL buffer and 0.2 µL of enzyme. To each PCR tube 5 µL of the enzyme mix was added. The incubation period was for 20 minutes at 60°C. The positive controls used in this PCR reaction was the cultivar ‘Pavon’ that showed a band size of 1361 bp. The negative controls used were the cultivars ‘Inia66’ and ‘Chinese Spring’ that showed a band pattern of 938 bp and 423 bp.

The enzyme *Fnu4HI* was added to the PCR left over products of the *TaGS5-3A* marker. The enzyme mix was made up of 4.4 µL water, 0.5 µL buffer and 0.1 µL of enzyme. To each PCR tube 5 µL of the enzyme mix was added. The incubation period was for 20 minutes at 37°C. The positive controls used for this marker was the cultivar ‘Opata’ that showed a fragment size of 863 bp. The cultivars ‘Pavon’ and ‘Chinese Spring’ were used as the negative controls and showed a fragment size of 718 bp.

3.3.4. Screening for the male sterility gene

The *Ms3* marker is associated with pollen sterility in wheat plants. The presence of this gene in the male sterile population was molecularly determined. One plant per pot, from the female population in the glasshouse, was randomly chosen to be screened for the presence of the male sterility gene. The PCR reaction mix contained 12.5 µL Red Readymix, 8.825 µL water, 0.625 µL *Ms3* forward primer, 1 µL *Ms3* reverse primer and 1 µL of DNA. The PCR conditions were as follow: denaturation for 5 minutes at 95°C, a duration of 35 cycle for 30 seconds at 95°C, 30 seconds at 58°C, 30 seconds at 72°C and a final extension for 7 minutes at 72°C. The PCR products were separated on 1.8% (w/v) agarose gels. The samples that showed positive amplification of the correct band size and had the correct phenotype in the glasshouse were used as positive controls. Water was used as a negative control.

Table 3.2. A list of Stellenbosch University Plant Breeding Laboratory's standard panel of markers.

Gene/ marker	**Primer**	**Primer sequence**	**Ta (°C)**	**Expected product size (bp)**	**Reference**
Sr2	CSSr2	F:5'-CAAGGGTTGCTAGGATTGGAAAAC-3'	60	53, 112, 172	Mago *et al.*, 2011
		R:5'- AGATAACTCTTATGATCTTACATTTTTCTG-3			
Sr24	719	F:5'-TCGTCCAGATCAGAATGTG-3'	57	719	Cherukuri *et al.*, 2003
		R:5'-CTCGTCGATTAGCAGTGAG-3'			
Lr37	VENT	F:5'-AGGGGCTACTGACCAAGGCT-3'	57	259	Helguera *et al.*, 2003
	LN2	R:5'-TGCAGCTACAGCAGTATGTACACAAAA-3'			
Lr19	12C	F:5'-CATCCTTGGGGACCTC-3'	57	119	Prins *et al.*, 2001
		R:5'-CCAGCTCGCATACATCCA-3'			
Lr34	Dint9	F:5'- TTGATGAAACCAGTTTTTTTTCTA- 3'	57	517	Krattinger *et al.*, 2009
	Lr34	R:5'- GCCATTTAACATAATCATGATGGA- 3'			
Sr26	Sr26#43	F:5'-AATCGTCCACATTGGCTTCT-3'	55	207	Mago *et al.*, 2005
		R:5'-CGCAACAAAATCATGCACTA-3'			
Sr31	Iag-95	F:5'-CTCTGTGGATAGTTACTTGATCGA-3'	55	1030	Mago *et al.*, 2005
		R:5'-CCTAGAACATGCATGGCTGTTCA-3'			
GluxDx	P1	F:5'-GCCTAGCAACCTTCACAATC-3'	60	450, 576, 612	Ahmad, 2000
	P2	R:5'-GAAACCTGCTGCGGACAAG-3'			
	P3	F:5'-GTTGGCCGGTCGGCTGCCATG-3'			
	P4	F:5'-TGGAGAAGTTGGATAGTACC-3'			
TaGW2	TaGW2-6B-CAPS	F:5'-GACTCCTCCTCGTCACCCATAAAGT-3'	64	1709	Qin *et al.*, 2014
		R: 5'-ATAGCACCAGCCCTTTCTCTTC-3'			
TaGS5	TaGS5-3A-CAPS	F:5'-AGACATGGTGGAGCAAGAGATG-3' R:5'-GAACAACCTAATCCTCCTCCTGA-3'	68	718, 863	Ma *et al.*, 2016

Gene/ marker	Primer	Primer sequence	Ta (°C)	Expected product size (bp)	Reference
Ppd-D1-2D	Ppd-D1-1	F:5'-ACGCCTCCCACTACACTG-3'	54	288, 4141	Beales *et al.*, 2007; Wilhelm *et al.*, 2013
		R:5'-GTTGGTTCAAACAGAGAGC-3'			
	Ppd-D1-2	R:5'-CACTGGTGGTAGCTGAGATT-3'			
TaGS-D1	GS7D	F:5'-AACTTAGGGAGCGAAAACAA-3'	52	522, 562	Zhang *et al*., 2014
		R:5'-CACCAAGACTGGAGATGAAA-3'			

Table 3.3. The simple sequence repeat markers that were used to screen for quantitative trait loci linked to Fusarium head blight resistance (Roder *et al*., 1998).

QTL	SRR marker	Primer sequence	Ta (°C)	Expected band size (bp)
Qhfs.ifa-5A	*gwm304*	F:5'- AGGAAACAGAAATATCGCGG-3' R:'5-AGGACTGTGGGGAATGAATG-3'	60	219
	gwm293	F:5'-TACTGGTTCACATTGGTGCG-3' R:'5- TCGCCATCACTCGTTCAAG-3'	60	207
7AQTL	*gwm130*	F:5'-AGCTCTGCTTCACGAGGAAG-3' R:'5-CTCCTCTTTATATCGCGTCCC-3'	60	126
	gwm233	F:5'-TCAAAACATAAATGTTCATTGGA-3' R:'5-TCAACCGTGTGTAATTTTGTCC-3'	50	288
Qfhs.ndsu-3BS	*gwm493*	F:5'-TTCCCATAACTAAAACCGCG-3' R:'5-GGAACATCATTTCTGGACTTTG-3'	60	211
	gwm533	F:5'-AAGGCGAATCAAACGGAATA-3' R:'5-GTTGCTTTAGGGGAAAAGCC-3'	60	160
	Barc133	F: 5'-AGCGCTCGAAAAGTCAG-3' R: 5'-GGCAGGTCCAACTCCAG-3'	60	125

3.3.5. Screening for *Fusarium* resistance

The population was screened for the major QTL's *Qfhs.ifa-5A, Qfhs.ndsu-3BS* and *7AQTL.* The QTL positions on wheat chromosomes are illustrated in Figure 3.2. The QTL's are linked to Type I, Type II and Type III FHB resistance. The markers *gwm304* and *gwm293* are closely linked to *Qfhs.ifa-5A* and associated with Type I FHB resistance (Buerstmayr *et al*., 2019). The QTL *Qfhs.ndsu-3BS* is flanked by the markers *gwm493, gwm533* and *Barc133* and is associated with Type II FHB resistance (Buerstmayr *et al.*, 2003). The *gwm130* and *gwm233* markers that are associated with Type II and Type III FHB resistance flanked the *7AQTL* (Jayatilake *et al*., 2011). A list of the markers are shown in Table 3.3.

The QTL's that conferred to FHB resistance in this study is flanked by two markers. The PCR reaction consisted out of 5.75 µL water, 6.25 Red Readymix, 0.5 µL forward and reverse primer. The PCR reaction had a total volume of 14 µL and the PCR reaction conditions were: the denaturation was at 94°C for 3 minutes, a duration of 44 cycles at 94°C for 1 minutes, for 1 minute at 60°C and 72°C for 1 minute which the final extension followed with 72°C for 7 minutes. The marker *gwm233* has an annealing temperature of 50°C. The negative control for this maker was the cultivar 'SST027' and water. The positive control the cultivar 'Sumai3' (FHB96). The positive and negative controls have different band sizes associated with the different markers and is showed in Table 3.3. The PCR products of the *gwm304, gwm293, gwm130, gwm233* and *Barc133* markers were visualized on 6% Polyacrylamide gels. The markers *gwm493* and *gwm533* band separation were visualized on 2% (w/v) agarose gels.

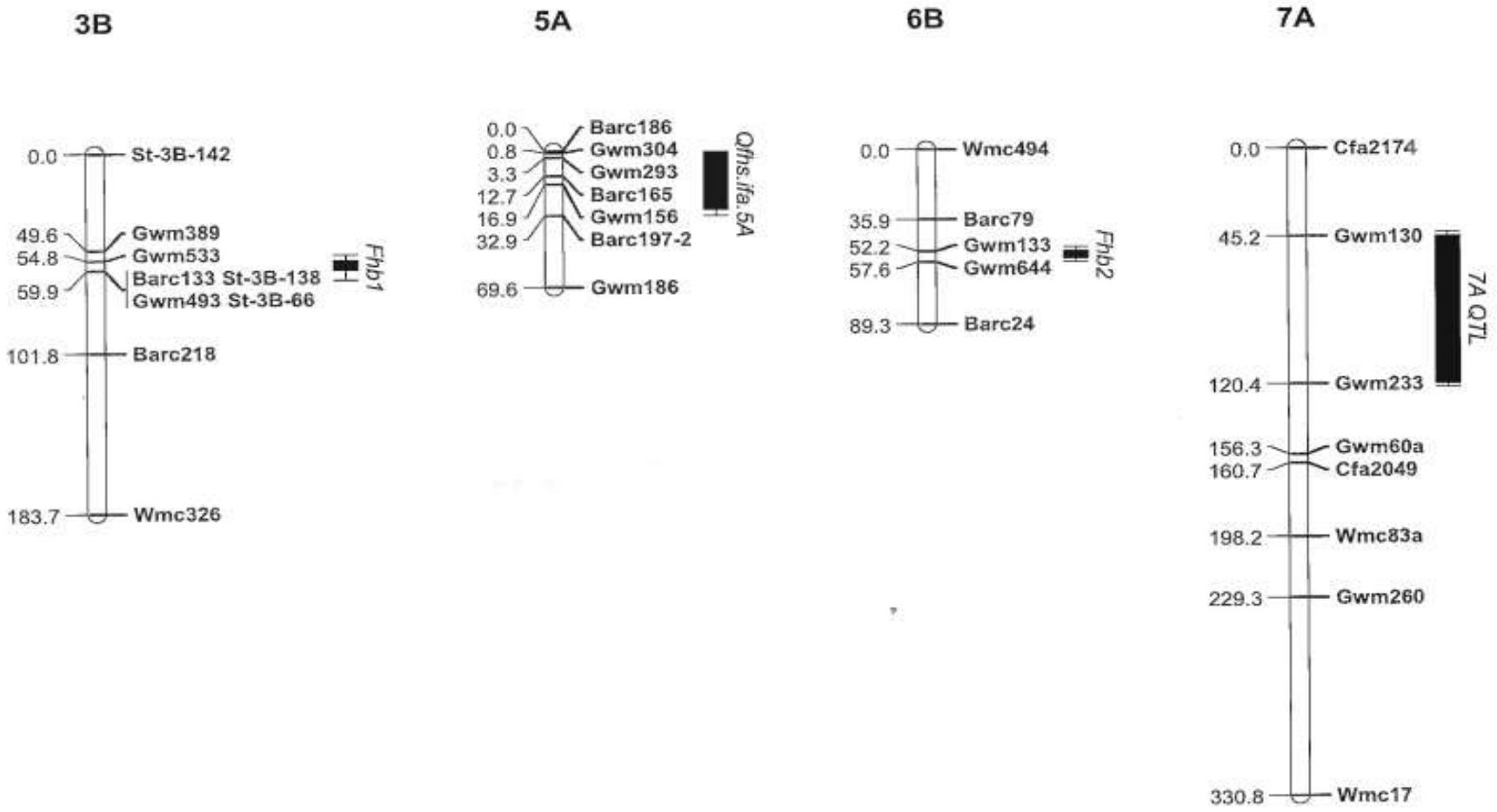

Figure 3.2. Illustrating some of the major FHB resistance quantitative trait loci positions on wheat chromosomes and the markers that covers the quantitative trait loci (Sydenham, 2014).

3.4. Agarose gels

To visualize the PCR products agarose gel electrophoresis were performed. Different agarose percentage gels were used for different markers. A 1%, 1,8%, 2% or 3% (w/v) agarose gels in 1X TBE buffer [5X TBE stock solution: 0.5 M Tris (hydroxymetyl) Aminomethane, 0.5 M Boric acid, 0.5 M EDTA were made up. The gels were stained with 4% (v/v) of EtBr and run at 120 V within 1X TBE buffer. The PCR products separation was visualized under a UV-light by using Uvitec gel imaging system (distributed by Whitehead Scientific Inc, Stikland, RSA).

3.5. Polyacrylamide gel- electrophoresis

The PAGE gel process are divided up into four steps namely plate preparation, gel preparation, loading of samples and silver staining.

3.5.1. Plate preparation

The plate preparation was done by using two 3 mm thick glass plates (long plates: 41.5 cm x 33 cm and shorter plates: 37 cm x 33 cm). Plates were cleaned using 70% ethanol. The long glass plate was cleaned using windscreen cleaner and left to dry for 3 minutes whereafter it was wiped off. A 125 µL of plate glue was diluted in 25 mL 100% ethanol and further diluted to a ratio of 1:3 containing 500 µL diluted plate glue and 1500 µL 100% ethanol. The shorter glass plate was wiped with 1740 µL diluted plate glue and 10% acetic acid. The glass plate was left to dry for 30 seconds then wiped off immediately. The plates were clamped together with a rubber and 1 mm spacers between the two glass plates.

3.5.2. Gel preparation

The preparation of the gel consisted out of 800 µL 10% [0.1 g APS dissolved in 1 mL dH_2O], 160 µL TEMED [N, N, N‟, N‟-Tetramethylethylenediamine] and 160 mL 6% sequencing gel. The 6% sequencing gel consisted of 37.5 mL 40% acrylamide stock solution (5.3 M acrylamide, 0.129 M bis-acrylamide and sufficient distilled water to a final volume of 200 mL), 90.09 g urea and 50 mL 5X TBE. The solution was mixed well before the gel was left for at least an hour to set.

3.5.3. Loading of samples

After the PCR 10 µL microsatellite loading dye [98 % (v/v) de-ionized formamide, 10 mM EDTA, pH 8.0, 0.05 % (w/v) bromophenol blue and 0.05 % (w/v) xylene cyanol FF] was added to each sample. Before loading the samples were denatured in a waterbath at 95°C for 5 minutes and kept on ice until 10 µL of each sample was loaded onto the gel. The GeneRuler 50bp and Biolabs 50bp ladders were used. The ladders consisted out of 0.9 µL loading gel, 0.9 µL ladder and 3 µL water. The gel ran at 70W for six hours.

3.5.4. Silver staining

After the separation of the plates the plate with the gel on it (long plate) was placed on a shaker (GFL 3016 horizontal shaker). To visualize the separation of the bands the gels were stained with silver staining. The staining process consisted of 20 minutes agitation on the shaker with the fixing solution [210 mL Ethanol, 10.5 mL Acetic Acid and 1879.5 mL dH_2O]. The gel was rinsed twice with 2 L dH_2O for 5 minutes. The gel was then stained using staining solution [2.1 $AgNO_3$ and 2100 mL dH_2O] for 20 minutes on the shaker. Following staining, the gel was rinsed with dH_2O for 10 seconds. The gel was treated with the developing solution [31.5 g NaOH, 2100 mL dH_2O and 8.505 mL Formaldehyde] and placed on the shaker until the bands appeared. After the gel was rinsed with dH_2O, a digital photo was taken.

3.6. Rust phenotyping

Based on the rust molecular data generated a selection of 250 wheat lines were planted at the Makhathini Experimental Station (MES) in KwaZulu-Natal (Figure 3.3). The trial was located in the northern parts of KwaZulu Natal that have warm and humid conditions (De Groot, 2012). Leaf rust and stem rust disease on wheat occurs in moist conditions with mild to warmer temperatures (Figueroa *et al*., 2018). The field trial location was ideally situated for evaluating stem and leaf rust resistance responses during the winter growth season (De Groot, 2012). Stripe rust disease on wheat occurs in cooler conditions (Figueroa *et al*., 2018), such as the wheat producing areas of the Eastern Cape. The location of MES does not have favourable conditions for stripe rust resistance evaluation in the field.

The field trial layout contained families that were planted together in blocks planted in 1 m rows. The trial also contained the cultivar 'SST806' that acted as a spreader which was planted throughout the trial. Spreaders are used for infection and uniform spreading of spores to cause disease epidemics. Spreaders are also used to extend the period of the epidemic by planting a mix of two to three susceptible cultivars (Velu & Singh, 2014). The wheat lines were inoculated twice with two leaf rust pathotypes, UVPt13 and UVPt21, and a stem rust pathotype UVPgt60. Spore suspensions were sprayed onto the wheat lines and spreader. The cultivar 'SST806' is susceptible to rust infection and was planted as a spreader in this field trial. Scoring of plant disease responses occurred 44 days after the last inoculation.

The adult plant responses were rated according to the Rust Scoring Guide, 1986. The field response were divided into six classes: (i) no visible infection (O), (ii) resistant (R) with visible chlorosis or necrosis, (iii) moderately resistant (MR) with small uredia present surrounded with some chlorosis or necrosis, (iv) intermediate (M) variable sizes of uredia are present surrounded by necrosis or chlorosis, (v) moderately susceptible (MS) where medium sized uredia are present and possible surrounded by chlorotic areas and susceptible (S) where large uredia are present with no necrosis and little or no chlorosis. A severity evaluation was also done together with the field response. The disease severity was scored as a percentage following the intervals of 10, 20, 40, 60 and 100. The disease severity was paired up with susceptible for

example 60% severity with a susceptible field response (60S). The leaf and stem rust plant disease responses were recorded early in the season.

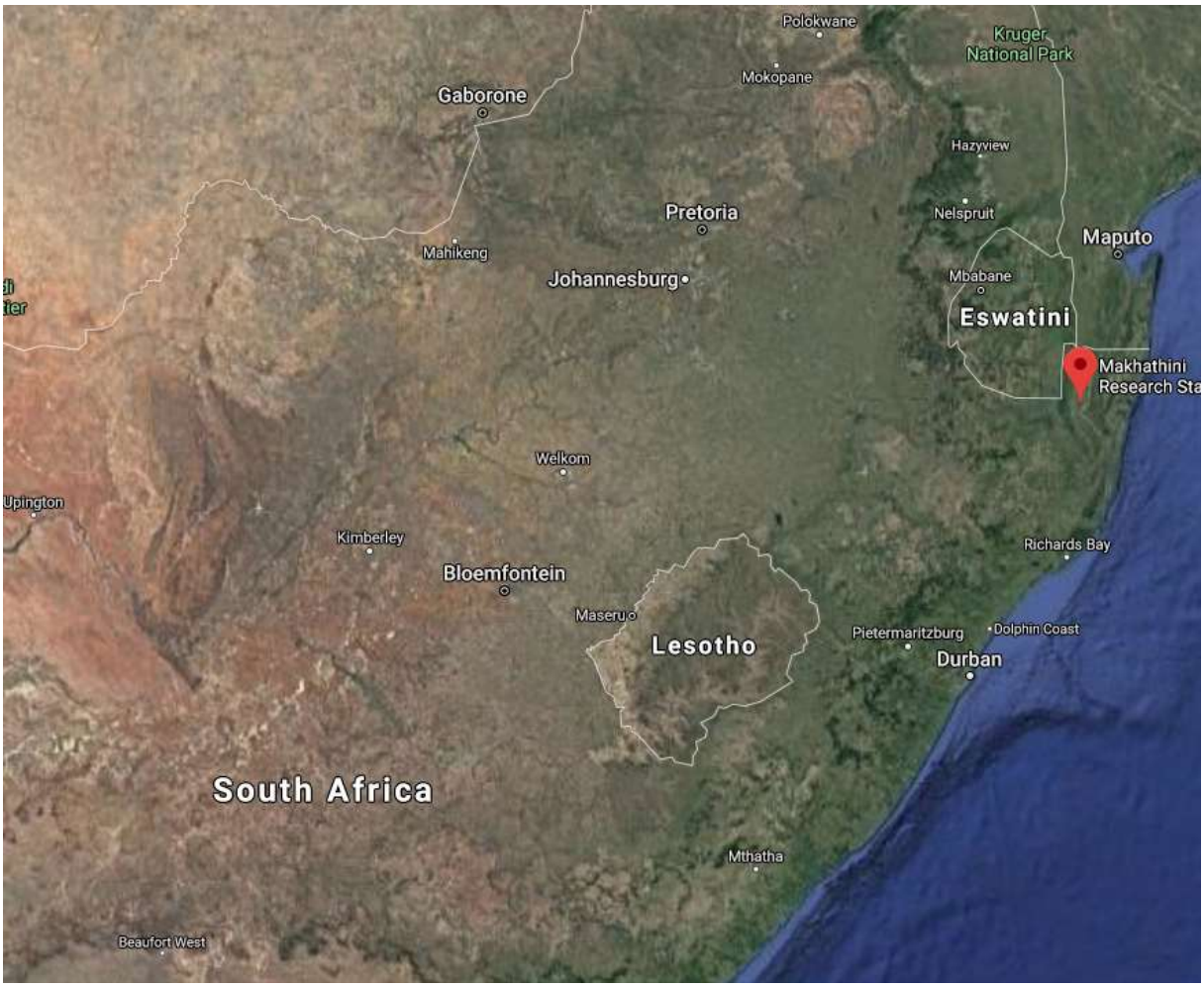

Figure 3.3. A map showing the eastern part of South Africa, displaying the location where the field rust phenotyping occurred, (Google maps [online] [Accessed 18 October 2020].

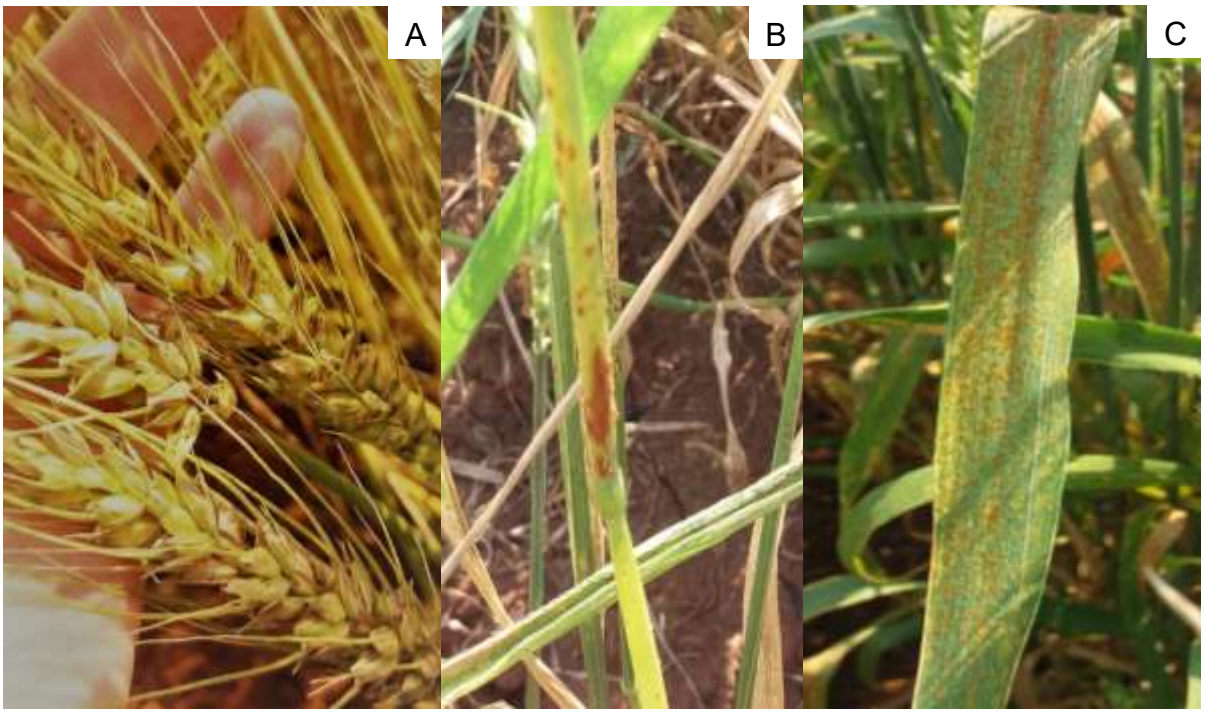

Figure 3.4. Visible rust disease on wheat plants at Makhathini Experimental Station. a) Wheat heads expressing the pseudo black chaff phenotype that is correlated to the stem rust resistance gene *Sr2*. b) Stem rust on a wheat stem. c) Leaf rust on a wheat leaf.

3.7. The MS-MARS pre-breeding scheme

The seeds of both female (male sterile) and donor/male population were planted in pots in the glasshouse. Four seeds of both male and female populations were planted per pot. The glasshouse was divided in half, separating the male and female populations. The glasshouse had four benches on each side and each bench contained 120 pots, of 3 L volume, filled with a mixture of course sand. The MS-MARS cycle one had a total of 420 pots that were used for both male and female populations. A total of 240 pots were used in MS-MARS cycle two to plant the male and female population. Seeds from the male population were planted on one bench each second week. Every other week seeds of the female population were planted. The planting period spanned over eight weeks.

3.7.1. Male sterility phenotyping

The female (male sterile) population contained the *Ms3* (male sterile) gene that segregates into 1:1 ratio of male fertile and sterile plants. The female plants that were molecularly screened for the *Ms3* gene was also phenotyped. The *Ms3* gene was linked to the sterile tillers having a light green and more open florets phenotype (Figure 3.5). The male sterile gene expression is unstable at high temperatures and therefore opening of florets was done to confirm sterility. The phenotypic data was compared to molecular data to determine if the *Ms3* marker was reliable.

3.7.2. Cross-pollinating events

At the flowering stage the sterile wheat tillers were cut twice a week and placed in water. The leaves of the tillers were removed except for the flag leaf. Cross pollination of the female tillers were increased by cutting the florets open (Figure 3.5). The tillers were then transferred to galvanized steel trays with a dimension of 600 mm x 450 mm x 160 mm (Figure 3.5) filled with a standard nutrient solution of 2 g Microplex (Ocean Agriculture Pty Ltd, Muldersdrift, South Africa), 164 g Sol-u-fert T3T (Kynoch Fertilizers Pty Ltd, Milnerton, RSA), 77 ml potassium nitrate in 100 L H_2O) and 0.05% Jik (household detergent containing 3.5% sodium hypochlorite, Reckitt and Colman South Africa Pty Ltd., Elandsfontein,RSA). The inside of the trays were painted black with antifungal paint.

The male population used in cross-pollination 2019 consisted of 30 wheat lines (F_6 population of the SUBPL's MS-MARS breeding program) that were selected based on molecular data generated. The donor population of MS-MARS cycle 2020 consisted of 26 selected wheat lines from the F_6 population and CIMMYT's nursery.

Just before the shedding of pollen the male tillers were cut. All the leaves were removed from the tillers and the tillers were placed in water. The tillers were then placed in two narrow galvanized steel trays that were 300 mm above the female tillers. The narrow trays were filled with the same standard nutrient solution and placed on both sides of the female tillers (Figure 3.4).

The pollination period of the male and female tillers was one week where after the male tillers were discarded. While the seed was ripening the nutrient solution of the female tillers was replaced every two weeks. The seeds were fully ripened after six weeks. The female tillers were placed in brown paper bags and dried at 20°C for a week in the oven. After a week the seeds were threshed. The total number of florets per ear and the amount of seeds threshed from each ear were counted. The threshed seeds acted as the F_2-female population of the next crossing season.

The rest of the female (male sterile) plants were left in the glasshouse to allow self-pollination and seed development. After the plant material matured and dried off, the amount of fertile and sterile plants were counted. The ratio between fertile and sterile plants per bench were determined. It was assumed that the female population would divide into a ratio of 1:1 of fertile and sterile individuals. Only one ear from a fertile plant was cut off and its seeds were threshed.

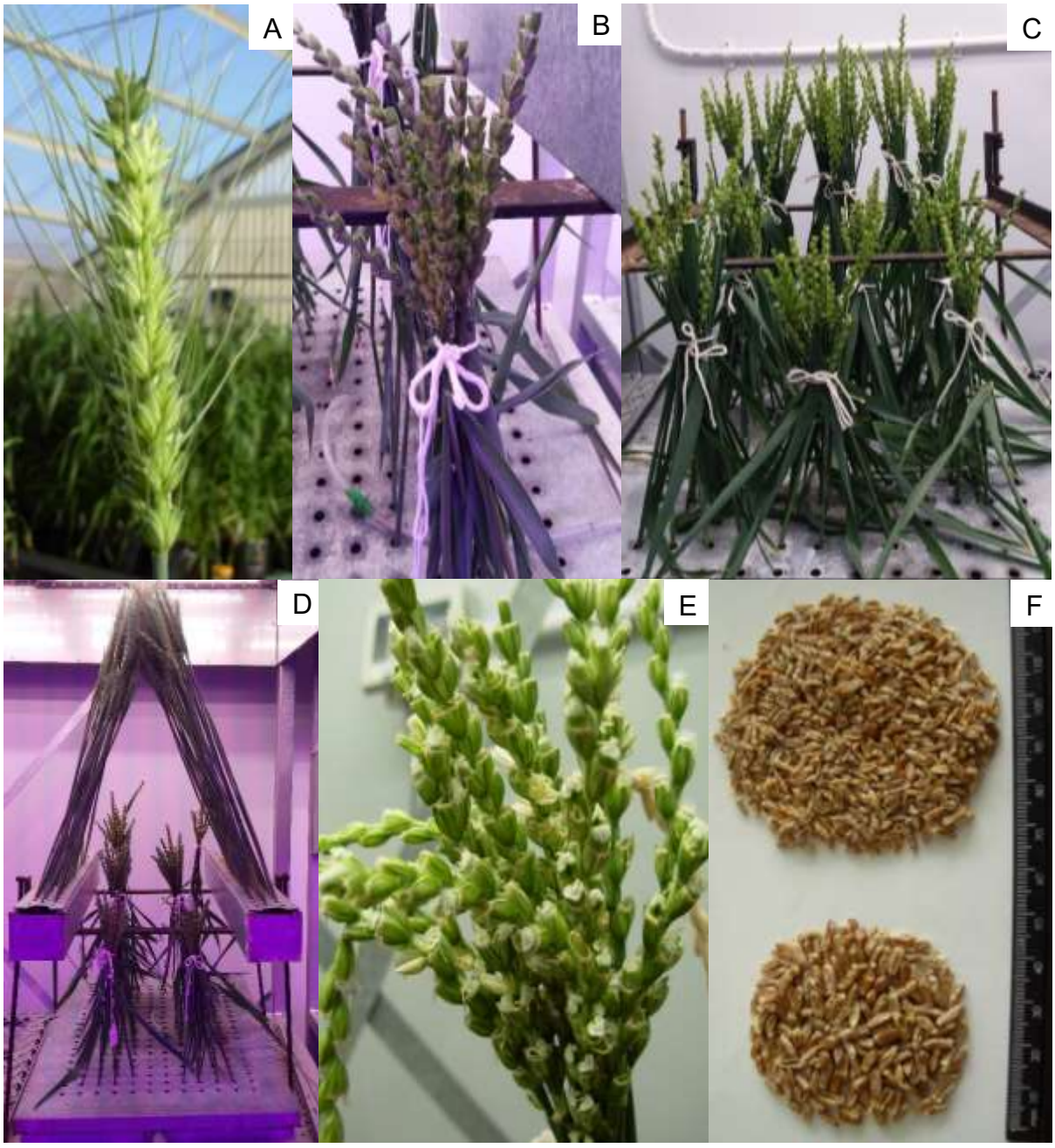

Figure 3.5. The cross pollination of wheat utilizing male sterility.

a) Phenotype of a male sterile tiller. b) Male sterile tillers that are cut open to enable cross pollination. c) Male sterile tillers that are cut open and placed in the hydroponic system. d) Male fertile tillers that are placed above male sterile tillers to pollinate. e) Seed development on sterile tillers. f) Four gram seed containing the male sterile phenotype (upper) versus 4 g of normal seed (lower).

3.8. Phenotyping of FHB

3.8.1. FHB phenotyping trial layout

A total of 56 wheat lines were planted and subjected to FHB resistance phenotyping during 2019. The wheat lines consisted of 29 of the SUPBL's F_6 population (selected based on the molecular data generated by screening the population with a standard panel of markers) and 27 of 21FHBSN_015 nursery. The two controls that were used in this experiment was the cultivars 'PAN3471' (susceptible) and 'Sumai3' (resistant).

Two types of resistance were tested for by making use of two different inoculation methods. The spray inoculation method was used to screen for Type I resistance while the point inoculation method was used to test for Type II resistance. The trail consisted out of two replicas of these 56 lines including the controls to perform both inoculation methods. For each inoculation method 116 pots (58 x 2) were planted. The glasshouse trail had a total of 232 pots, where four seeds per pot were planted. The temperature and humidity were measured during the anthesis period throughout the kernel development stages of wheat.

The FHB phenotyping trial during 2020 consisted out of 13 wheat lines from the SUPBL's F_6 population and 13 lines from the 21FHBSN_015 nursery that were planted in three replicates. The lines were selected based on performance in the FHB phenotype trial of 2019. Each wheat line had a water control pot which represented a negative control replicate. The cultivars 'Sumai3' and PAN3471' was again used as resistance and susceptible controls. A total of 112 pots were planted with nine seeds per pot. The point inoculation method was used to screen the wheat lines for Type II resistance. The temperature and humidity were also measured throughout the trial.

3.8.2. Growth of cultures

Three sub-cultured, single spored *F. graminearum* isolates (Table 3.4) obtained from Dr G.J. van Coller (Western Cape Department of Agriculture) were plated out on PDA

[2% Potato Dextrose Agar (Biolab, Midrand)] media and stored at 25°C. The production of macroconidia were induced by the plating isolates on carnation leaf agar [Biolab Diagnostics (Pty) Ltd, Midrand, South Africa] to stimulate sporulation. Each petri dish contained five carnation leaves. Spores were produced in about 2-3 weeks. Additionally, the production of macroconidia were also induced by adding a Cu-Zn solution (10 mM $CuSO_4$ + 10 mM $ZnSO_4$) to fungal growth on a PDA plate. Filter paper were dipped in the Cu-Zn solution and placed in the middle of a PDA plate where macroconidia were produced after three days (Figure 3.6).

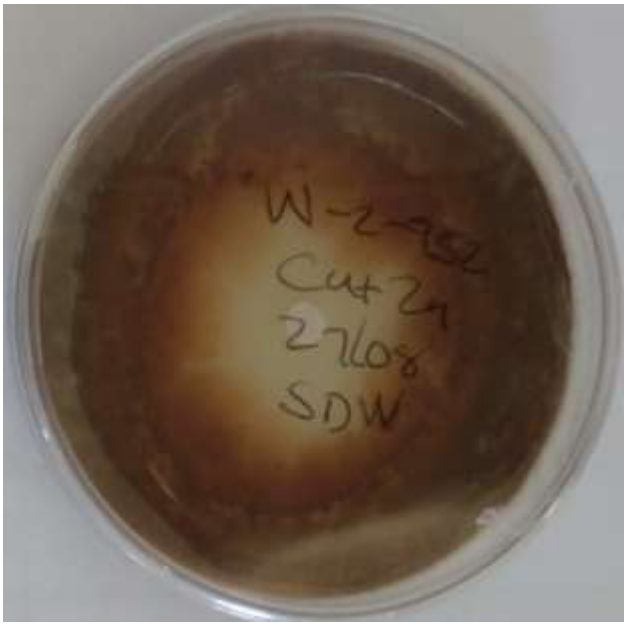

Figure 3.6. *Fusarium graminearum* on potato dextrose agar, producing macroconidia after a filter paper was dipped in a Cu-Zn (10 mM) solution and placed on the petri dish.

Table 3.4. *Fusarium graminearum* isolates used to infect wheat plants under glasshouse conditions to screen for resistance to Fusarium head blight of wheat.

Isolate Number	Host	Cultivar	Province	Locality	Year	Chemotype
W-2-922	Wheat	Kariega	Northern Cape	Bull Hill	2009	15-ADON
W-2-952	Wheat	Baviaans	Northern Cape	Hopetown	2009	15-ADON
W-2-962	Wheat	Kariega	Northern Cape	Barkley-Wes	2009	15-ADON

3.8.3. Preparation of inoculum

Spore suspensions for each isolate was prepped by adding 5-7 mL of autoclaved water onto petri dishes treated with the Cu-Zn solution. A microscope glass slide was used to scrape off spores and mycelia. Petri dishes that contained carnation leaves were scraped with a hockey stick. The mycelia and spores were filtered through sterile cheesecloth (Figure 3.7). These steps were repeated multiple times for each isolate. The spore concentration of each isolate was determined by pipetting 10 µL of spore suspension onto the haemocytometer. The upper and lower 5 blocks of the haemocytometer's middle block was used to count spores under a light microscope (Figure 3.8).

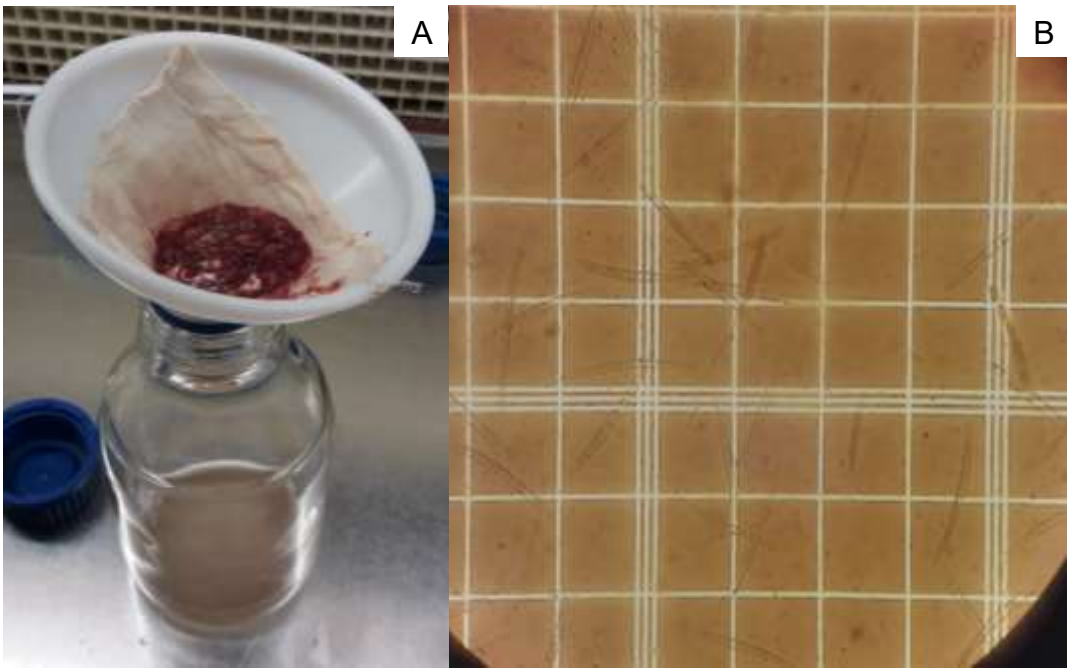

Figure 3.7. Harvesting spores from the cultures.
a) Harvesting macroconidia through cheesecloth. b) Macroconidia spores under the light microscope at 410X magnification.

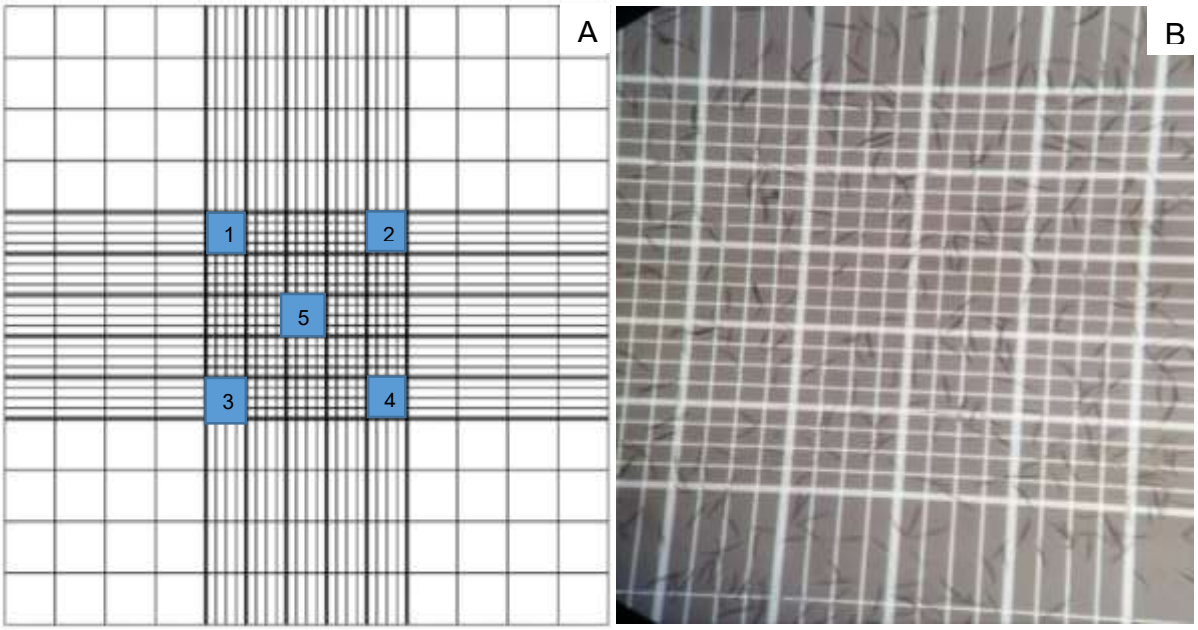

Figure 3.8. Macroconidia spore concentration of *Fusarium graminearum* plates. a) Blocks of the haemocytometer that were used to determine the macroconidia spore concentration for *F. graminearum* isolates. b) Macroconidia spores under the light microscope at 110X magnification.

The spore concentration (spores per mL) of each isolate was calculated by the average of the lower and the upper block of the haemocytometer times 50 000. Dilution calculations were applied for each isolate by using the $C_1V_1 = C_2V_2$ equation. The required spore concentration needed for inoculation was 50 000 spores per mL. Equal volumes of the different isolate suspensions were added together to make up the total volume needed for each inoculation method.

3.8.4. Inoculation

The amount of inoculum was prepared based on the number of ears that were ready to be inoculated was counted. When 50% or more of a pot's ears were shedding pollen at anthesis, the pot was chosen for inoculation. Inoculum was prepared the day before inoculations and stored at 4ºC. Ears that were ready for infection had visible yellow anthers outside of the floret. The anthers of the ears should still be yellow and not white/light yellow colour. No opening of florets occurred. Spore suspensions were kept on ice at all time once removed from 4°C storage and mixed again prior to inoculation. The inoculation process spanned over two weeks that were divided in five inoculation batches during the FHB phenotype trial of 2019. The inoculation process of FHB phenotype trials in 2020 spanned over four weeks that were divided in seven inoculation batches (2020).

3.8.4.1. Spray inoculation method

The wheat ears were held together and sprayed twice with spore suspension, once in front and once at the back. Spray inoculations were performed by using a spray bottle that dispersed 1 mL at a time. The inoculated ears were then covered with a plastic zip lock bag closed tightly with a ribbon to increase humidity around the inoculated wheat head. A brown paper bag was used to cover the plastic bag to protect the inoculation from sunlight. After 24 hours all the bags covering the ears were removed. The spray inoculation method is shown in Figure 3.9.

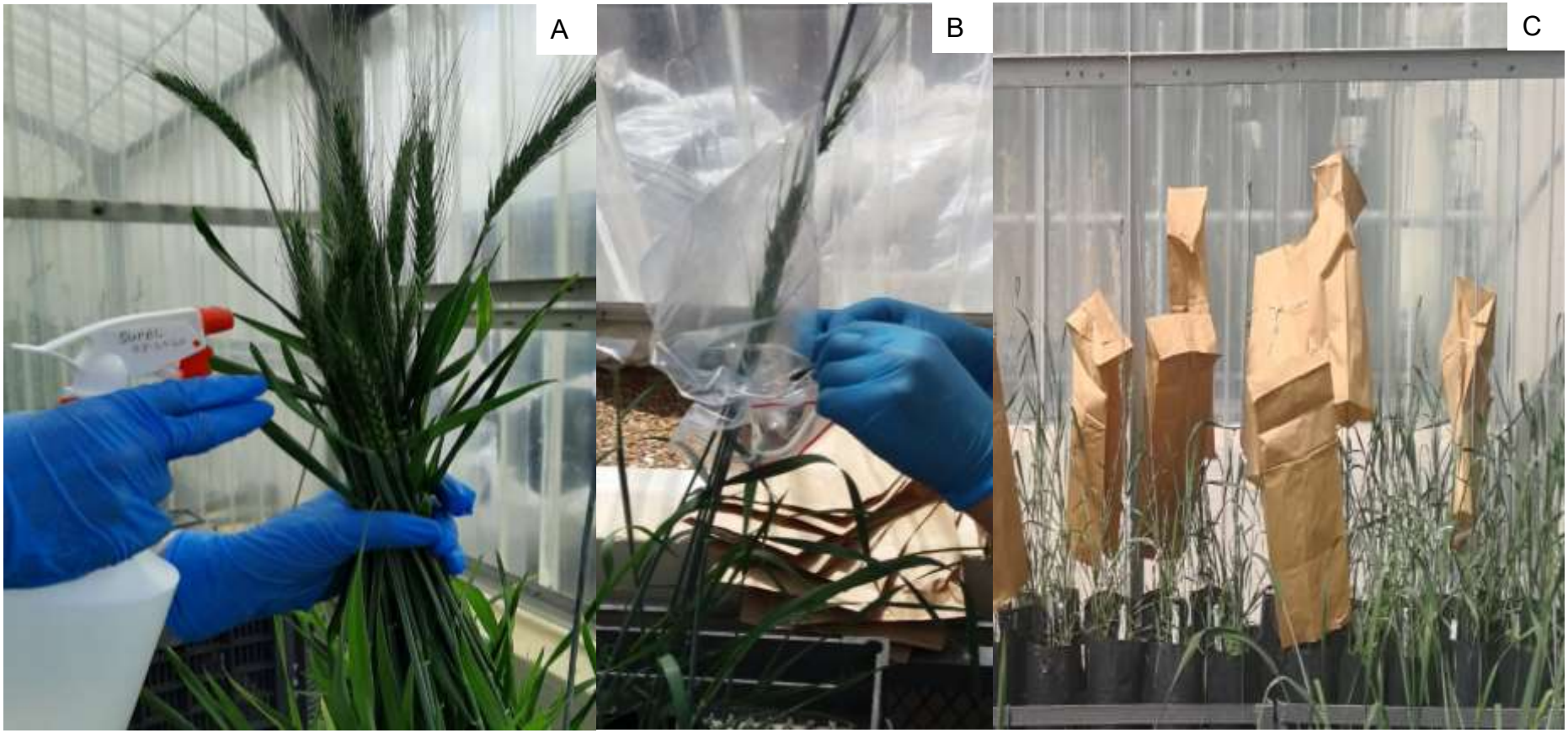

Figure 3.9. Spray inoculation method.

a) Spraying a cocktail of *Fusarium graminearum* isolates on wheat heads. b) The inoculated ears were covered with a plastic bag to create humidity. c) A brown paper bag covered the plastic bag to protect the wheat heads from sunlight exposure.

3.8.4.2. Point inoculation method

The centre floret inoculation of wheat ears affects the movement of nutrients and water to the top florets (Masri *et al.*, 2017). This proved that the downward colonisation of *F. graminearum* is stronger compared to the upwards colonisation. The point inoculations were done by pipetting 20 µL of spore suspension in the second flower from the top of a wheat ear. The point of inoculation was marked with a black permanent marker and after the ears were inoculated it was covered with bags tightly closed with a ribbon that helped to create a humid chamber. The pot with inoculated ears were then placed in a dew chamber for 72 hours at a temperature of 20 to 25°C with 100% RH. After three days the bags were removed from the ears, the ears were tagged and the pots were placed back inside the glasshouse. The point inoculation method is shown in Figure 3.10.

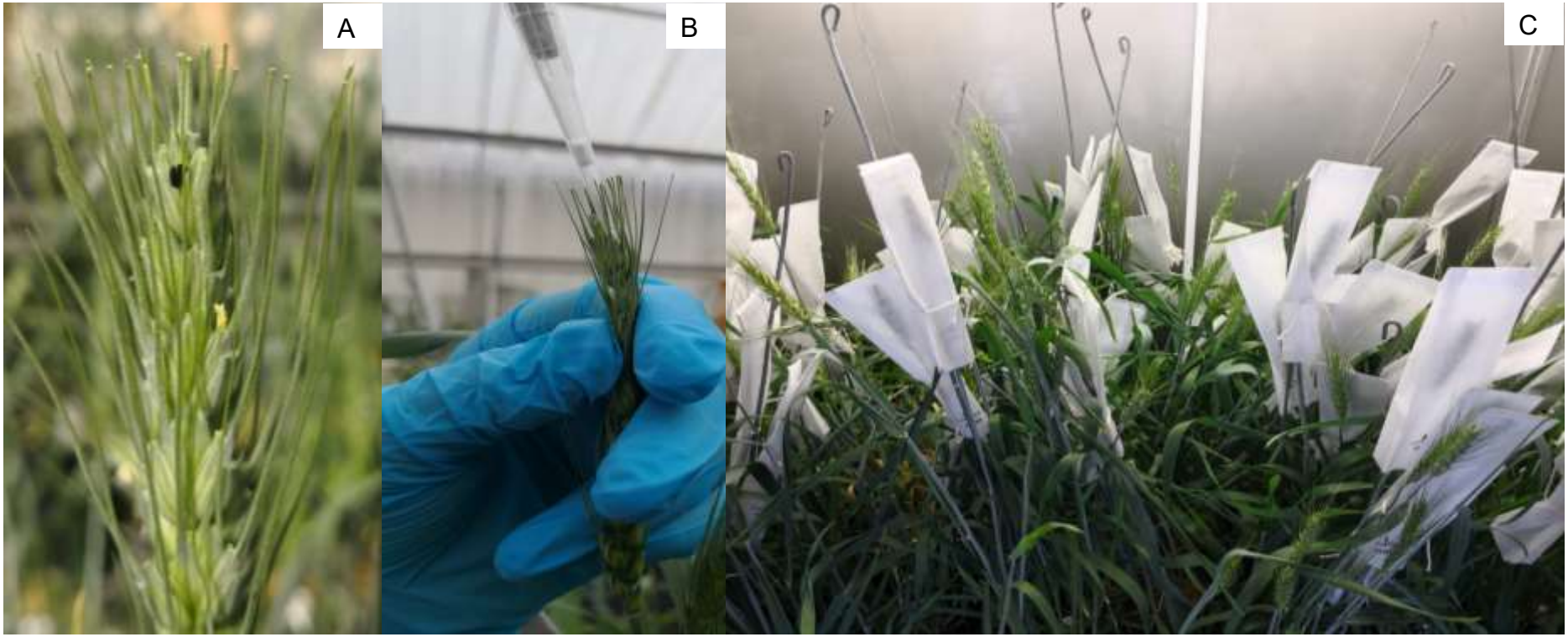

Figure 3.10. Point inoculation method.

a) The point of inoculation marked on a wheat head. b) Inoculating an ear by pipetting a cocktail of *Fusarium* species into the second floret from above. c) Inoculated ears are covered with a bag to create humidity and placed in the dew chamber.

3.8.5. FHB phenotyping measurements

3.8.5.1. Incidence and severity

The scoring of FHB resistance responses occurred on 14, 21 and 28 days post inoculation (dpi) for both inoculation methods. The disease severity was rated by the following equation: (total number of infected florets ÷ total number of florets) x 100 = %. The average disease severity of wheat lines were calculated for both inoculation methods. At 21 dpi photos were taken of bleached wheat heads to visualize disease progress. Graphs were drawn up to show disease severity and disease progress of the different wheat lines as well as the two inoculated methods that were applied. The least significant difference (LSD) 5% values of the data generated was calculated to determine significant differences between lines and the controls used in the experiment. Data analysis were performed by using Agrobase© Generation II version 34.4.1 (Agronomix® Software, Winnipeg, Canada) on results collected from the FHB phenotyping trials. A three factorial data analysis were performed on the average disease severity data generated from FHB phenotyping trial 2019. A two factorial data analysis were performed on data collected from the FHB phenotyping trial 2020.

3.8.5.2. Mycotoxin analysis

A total of 88 samples consisted of 23 wheat lines that were tested for the presence of DON, NIV and ZEA contamination in seeds. The wheat lines were infected with *F. graminearum* isolates during anthesis and were left to mature and dry off. The seeds were per pot was pooled and threshed. The seeds were milled with a Perten mill feeder 3170 and 5 g of flour were weight off in 50 mL Falcon tubes.

The mycotoxin extraction was done by adding 20 mL extraction buffer that consisted out of 70% Methanol (Microsep, Sandton, RSA) and 30% water (HPLC-grade) to the milled grain. The extraction buffer was added to samples at a 4:1 ratio to sample weight. The suspended samples were placed a shaker-incubater for 30 minutes at 200 rpm at 25°C. The samples were then centrifuged for 10 minutes at 500

rcf at 4°C. The supernatant was filter sterilised using 0.22 µM recombinant cellulose filters directly into 2 mL microcentrifuge tubes. Samples were refrigerated overnight, centrifuged at 13 000 rpm for 10 minutes and the supernatant transferred to analytical vials for analyses. Six standards with different concentrations of Trichothecenes (TCTs) and ZEA as well as a blank sample consisted only of 70% methanol was included for analysis (Table 3.5). The samples were then submitted to the Mass Spectrometry unit at the Central Analytical Facility (CAF) at Stellenbosch University. The mycotoxin analyses were done using liquid chromatography tandem mass spectrometry (LC-MS/MS).

Table 3.5. The standard concentration range of deoxynivalenol, nivalenol and zearalenone used as reference standards for different mycotoxin analyses.

Standard name	TCT (DON, NIV) (ppb)	ZEA (ppb)
STD 1	0.032	0.064
STD 2	0.160	0.32
STD 3	0.800	1.6
STD 4	4.000	8
STD 5	20.000	40
STD 6	100.000	200

Chapter 4: Results and Discussion

4.1. Molecular characterising

4.1.1. Screening the male (pollen donor) population

The donor population consisted out of selected lines from the 2018 F_6-SUPBL population and CYMMIYT's 21FHBSN015 nursery. The wheat lines were screened with the SUPBL's standard panel of markers that consisted out of rust resistance, baking quality, yield and FHB resistance markers (Addendum A). The rust resistance gene frequencies of the pollen donor populations of both MS-MARS cycles were calculated (Table 4.1). The gene frequencies of MS-MARS cycle 1 were not exceptionally high. This could be explained by the selection of only 4.82% of the total male population for the cross-pollination events based on the combined molecular data generated. The rest of the 95.18% male population may contain higher favourable gene frequencies not present in the tested population.

The rust resistance gene frequencies of *Sr2, Lr34, Sr24* and *Sr31* were lower in the MS-MARS cycle 2. The lower gene frequency could be explained by the selection of only 3.99% of the total male population for cross-pollination events. The selection was based on the performance of wheat lines in the 2019 FHB phenotyping trial and not on the presence of favourable genes in the genetic backgrounds. The rest of the 96.01% of the male population may obtain higher favourable gene frequencies. The leaf rust resistance, *Lr37*, gene frequency increased with 11.67% from MS-MARS cycle 1 to 2.

The *Sr26* gene had a gene frequency of 0% which reflected that it was a newly introduced gene into the MS-MARS breeding program. Several recurrent selection cycles are needed to establish a newly introduced gene in the breeding program and to increase desirable gene frequencies (Marais and Botes, 2009). It was also reported that four recurrent selection cycles are needed to significantly increase low gene frequencies of 5%.

The gene frequencies of the QTL's linked to FHB resistance were calculated to be 0% in the donor populations of both cycles. Historically, the SUPBL's breeding scheme mostly focused on improving rust resistance and yield of wheat material. Recently, the focus shifted to breeding for resistance against multiple diseases including FHB resistance. Most of the FHB resistance sources originated from China, where FHB epidemics were frequently experienced (Ma *et al.*, 2020). The germplasm and nursery that was used in this study were adapted to South African and Mexico climates. This contributed to the low QTL (FHB resistance) frequencies present in the donor populations. FHB resistance is also known to be a very complicated trait because it's controlled by multiple QTL's with small effects and no single gene results in complete resistance (Dong *et al.*, 2018; Yi *et al.*, 2018). In this study the donor population were only screened for three major QTL's namely *Qfhs.ifa-5A* (chromosome 5AS), *7AQTL* (chromosome 7A), *Qhfhs.ndsu-3BS* (chromosome 3BS). The absence of these QTL's in the population does not result in total susceptibility because the lines may contain other multiple or smaller QTL's linked to resistance that were not screened for.

Table 4.1. Rust resistance gene frequencies of the donor population.

Target genes	MS-MARS cycle 1 (2019)	MS-MARS cycle 2 (2020)
Sr2	16.67%	5.00%
Lr34	30.00%	20.00%
Sr24	43.33%	35.00%
Lr37	13.33%	25.00%
Sr26	absent	absent
Sr31	16.67%	15.00%

Virulence have been reported against stem rust resistance genes *Sr24* and *Sr31*. Virulence was detected during Ug99 race studies and was the first race to show virulence against the widely deployed *Sr31* gene. Virulence against the leaf rust resistance gene *Lr37* have also been reported since new *Pt* races were detected in South Africa in 2008 (Pretorius *et al.*, 2020). The *Sr24, Sr31* and *Lr37* genes are

linked to other rust resistance genes that form gene clusters. The gene clusters *Sr24/Lr24, Sr31/Lr26/Yr9/Pm8* and *Lr37/Yr17/Sr38* can still contribute to pyramiding resistance genes to achieve more durable resistance (Helguera *et al.*, 2003; Mago *et al.*, 2005; Aktar-Uz-Zaman *et al.*, 2017). No virulence against *Sr26* have been reported and remains effective against the Ug99, TTKST and TTTSK variants (Aktar-Uz-Zaman *et al.*, 2017). No virulence has been detected against the *Sr2* and *Lr34* rust resistance genes. These APR genes maintains moderate levels of resistance during rust field epidemics (Pretorius *et al.*, 2020).

4.1.2. Molecular screening of female (male sterile) populations

The segregating F_0 and F_1 base populations (female populations) of both MS-MARS cycles were screened for the presence of slow rusting genes *Sr2* and *Lr34*. Only 28.57% (120 plants) of the total female population were molecularly screened. The F_0-population had gene frequencies of 28.33% and 25.83% for *Sr2* and *Lr34* rust resistance genes (Figure 4.1.). The F_1-population had *Sr2* and *Lr34* rust resistance gene frequencies of 35.83% and 30.00%. The results showed that there was an increase in the gene frequencies from the F_0 to F_1 population after the cross-pollination events. The gene frequency of *Sr2* increased with 1.67%, while the *Lr34* gene frequency increased by 10.00%. The increased gene frequencies illustrate the effectiveness of recurrent selection in the MS-MARS breeding scheme. According to Marais and Botes (2009) gene frequencies of dominant alleles will change gradually after several recurrent selection cycles. Further increases of desirable gene frequencies can be expected in the next MS-MARS cycle. The *Sr2* gene had a lower increase, compared to *Lr34*. This is because the gene frequency of *Sr2* was lower in the donor population of MS-MARS cycle 2. Marais and Botes (2009) reported that continuous positive selection for a gene results in expected increase of allele frequency over successive cycles.

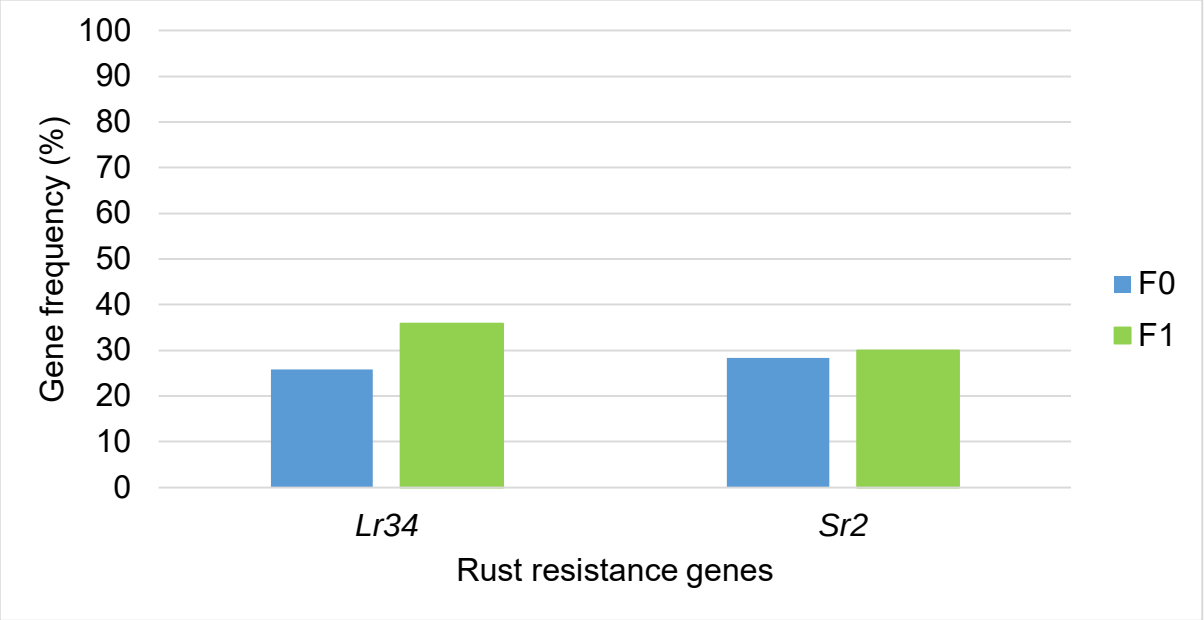

Figure 4.1. Wheat rust resistance gene frequencies of the female population before and after cross-pollinations.

4.2. Recurrent MS-MARS pre-breeding scheme

4.2.1. MS-MARS cycle 1 (2019)

The cross-pollination event occurred between the 2018 F_6-SUPBL's population and the segregating F_0 base population. A total amount of 1307 male fertile plants and 1137 male sterile plants were sourced from the donor and the female populations over 12 cutting sessions. A total number of 3919 seeds were harvested in 12 harvesting periods. The harvested seeds were small, shrivelled and had a total kernel weight of 13.6 g. Hybrid seed harvested from successful cross-pollination have a shrivelled, small phenotype (Marais and Botes, 2009). According to Marais *et al.* (2001) the seed harvested after pollination were relatively small and 80-90% of the seeds have the ability to germinate. The overall average percentage successful cross-pollination of MS-MARS cycle 1 was 42.93% (Table 4.2).

4.2.2. MS-MARS cycle 2 (2020)

Thirteen wheat lines from both the 2018 F_6-SUPBL's population and CYMMIT's 21FHBSN015 nursery were included as donor lines in the cross-pollination events. The seeds sourced from MS-MARS cycle 1 was used as the F_1 base population (sterile male population) to facilitate mass crossings. A total number of 677 fertile plants were sourced from the donor population and a total amount of 756 sterile plants were collected from the female population over 14 cutting sessions. During 14 harvested periods a number of 9716 seeds were harvested with a total kernel weight of 6.6 g. The overall average percentage successful cross pollination was 43.64% (Table 4.3).

The overall average percentage successful cross pollination increased with 0.74% from MS-MARS cycle 1 to cycle 2. The low successful cross-pollination can be explained by the fact that wheat has a natural overall low crosspollination rate (lower than 1%) because it's a strong self-pollinating plant. Self-pollination is also promoted by fertilization occurring before the florets open known as cleistogamy (closed pollination) (Rieben *et al.*, 2011; Okada *et al.*, 2018). The cytoplasmic male sterility gene (*Ms3*) used in this study promotes cross pollination and becomes unstable at temperatures higher than 21°C (Maan & Williams, 1984). A data logger measured temperatures in a nearby glasshouse that recorded temperatures of as a high as 55.59°C in cycle 1 and 56.8°C in cycle 2 (Figure 4.2 and 4.10). These recorded temperatures are much higher than the *Ms3* gene stability threshold. The extreme heat plants experienced during anthesis in the glasshouse explains the low average cross-pollination rate and that self-pollination were promoted.

The total kernel weight of seeds harvested in MS-MARS cycle 2 were lower than in cycle 1. According to a study done by Marais *et al*. (2001) the seed set on male sterile tillers are usually low. Another explanation for the lower seed weight was the high glasshouse temperatures experienced and limited glasshouse space during MS-MARS cycle 2 because it was shared with other students.

Table 4.2. MS-MARS cycle 1.

Crossing event	Date of cuttings (2019)	Total amount of female plants	Total amount of male plants	Maximum possible unique combinations	Number of seeds harvested from sterile plants	Average cross pollination (%)
1	16/09	32	51	1632	424	48.69
2	19/09	60	99	5940	348	39.36
3	23/09	58	83	4814	401	67.45
4	26/09	55	95	5225	337	37.52
5	30/09	51	137	6987	400	49.67
6	03/10	35	121	4235	445	56.48
7	07/10	143	134	19162	330	34.80
8	10/10	182	133	24206	324	47.92
9	14/10	227	119	27013	231	30.65
10	17/10	180	144	25920	314	45.69
11	21/10	76	128	9728	247	39.89
12	28/10	38	63	2394	118	17.07
	Total	1137	1307	137256	3919	42.93

Table 4.3. MS-MARS cycle 2.

Crossing event	Date of cuttings (2020)	Total amount of female plants	Total amount of male plants	Maximum possible unique combinations	Number of seeds harvested from sterile plants	Average cross pollination (%)
1	07/09	20	7	140	266	43.53
2	10/09	28	34	952	382	41.16
3	14/09	56	60	3360	893	47.11
4	17/09	44	60	2640	822	62.67
5	21/09	68	61	4148	1102	53.01
6	24/09	72	57	4104	1072	51.05
7	28/09	80	53	4240	616	29.80
8	01/10	72	51	3672	1168	62.65
9	05/10	66	55	3630	901	43.05
10	08/10	70	53	3710	962	45.33
11	12/10	44	53	2332	517	38.02
12	15/10	43	50	2150	362	30.94
13	19/10	43	42	1806	287	24.74
14	21/10	50	41	2050	366	37.92
	Total	756	677	38934	9716	43.64

4.2.3. Inheritance of sterile male gene

The sterile male plants of both MS-MARS cycles were selected based on the phenotypical presence of the dominant male sterile gene, *Ms3*, located on chromosome 5AS. The typical phenotypic traits of a male sterile tiller is a lighter green colour, more open florets and small anthers with lack of pollen. The male sterile (*Ms3*) gene is penetrable resulting in tillers having the male sterile phenotype but containing anthers that are bigger and fertile when opening the florets. The inheritance of the *Ms3* gene in the segregating male sterile populations, used for cross-pollinations, were determined via chi-square analysis. After cuttings of male sterile and male fertile tillers of the female population were counted the chi-square tests were conducted for each female population bench in both MS-MARS cycles. The female population, that contains the *Ms3* gene, will segregate into a 1:1 ratio of male fertile versus male sterile plants according to a study done by Marais *et al*. (2001). The p-value of the chi-square analysis for MS-MARS cycle 1 and 2 were greater than 0.05 (Table 4.4. and 4.5.). This indicated that benches one to four showed good fit to the 1:1 ratio in both cycles. The overall p-value of both cycles were also calculated as greater than 0.05 and thus does not deviate from the 1:1 segregating ratio. The results confirm the presence of the single dominant male sterility gene in the female population.

Table 4.4. Displaying the inheritance of sterile gene in MS-MARS cycle 1.

Bench number in glasshouse	Total number of sterile plants	Total number of fertile plants	Total number of plants	X^2	Probability to fit 1:1 ratio
1	253	212	465	1.808	0.405
2	249	238	487	0.124	0.940
3	225	215	440	0.114	0.945
4	75	62	137	0.617	0.735
Total	802	727	1529	1.839	0.399

Table 4.5. Displaying the inheritance of sterile gene in MS-MARS cycle 2.

Bench number in glasshouse	Total number of sterile plants	Total number of fertile plants	Total number of plants	X^2	Probability to fit 1:1 ratio
1	129	160	289	1.663	0.435
2	79	98	177	1.020	0.601
3	117	102	219	0.514	0.773
4	23	23	46	0.000	1.000
Total	348	383	731	0.838	0.658

To phenotypically identify male sterile tillers in the female population is labour intensive, time consuming and can result in wrong identification when the *Ms3* gene becomes unstable. By using a marker to identify sterile plants within the female population would result in saving time and promote cross-pollination. The female population of MS-MARS cycle 2 were screened for the presence of the male sterility (*Ms3*) marker (Addendum B). A total of a 120 plants were molecular screened and the gene frequencies of the *Ms3* gene in the female population was calculated as 41.67%.

The same 120 plants were phenotypically screened and scored as either fertile or sterile (Addendum B). The phenotype data recorded 43.33% sterile plants and 54.17% fertile plants within the population. The molecular data and phenotype data collected from 50 wheat lines in this study had contradictory results. This can be explained by the high glasshouse temperatures recorded (between 6.8°C and 56.8°C) by a data logger in a nearby glasshouse during the anthesis growth stage (Figure 4.10). It was reported by Maan & Williams (1984) that the *Ms3* gene expression becomes unstable at temperatures higher than 21°C. This could have resulted in the imbalance of sterile plants versus fertile plants. Another explanation for the observed results could be that the *Ms3* marker used in this study is not reliable and doesn't amplify the correct sequence representing the *Ms3* sterility gene. A study done by Liu *et al.* (2012) stated that markers genotype-phenotype needs to be determined when used in high-throughput screening.

Previous studies that worked on the MS-MARS female population also didn't find a 1:1 sterile vs fertile phenotype ratio. In 2014 the female population had 47.06% sterile plants and 52.94% fertile plants according to the study done by Springfield, (2014). In 2017 the female population consisted out of 45.00% fertile plants and 54.99% sterile plants (Meintjies, 2017). Rhoda (2018) also reported the female population to have 43.43% fertile plants and 56.57% sterile plants.

4.3. Rust phenotyping

The spreaders that were susceptible to stem rust showed no symptoms of disease development, therefore no data could be reported. Some of the other wheat lines in the trial showed stem rust disease symptoms, indicating the pathogen was present in the field but not yet in high severity. The timing of stem rust resistance phenotyping could be too early and that infection within the field has not yet been established or the conditions were not optimal for disease development. Evaluation of stem rust resistance responses on the wheat lines could not be recorded later in the season because of logistical problems and the overlapping of the occurrence of another trial in this study.

The cultivar 'SST806' showed phenotypic responses of 60MS, 60S, 20S and 40S to leaf rust (Addendum C), indicating that disease infection was well established in the field. According to Velu & Singh (2014) the acceptable degree of resistance needs to be decided and then plants will only be selected that shows at least that level of resistance. It was decided for this study that a 60S response to leaf rust is too high, showing that either the line did not contain leaf rust resistance genes or the resistance genes are ineffective. The families that contained wheat lines that showed a 60S response were not selected for future rust resistance breeding.

The families that contained wheat lines with MS or MR responses were also not selected for future rust resistance breeding. According to Singh and Rajaram (2002) MR phenotype can represent only major resistance genes in a genetic background. Major genes are classified as race specific resistance genes and are recognised by low infection phenotypes. These major genes are vulnerable to pathogens overcoming the resistance even when the major genes are singular or in combinations.

Breeding with only these major genes in a genetic background is not practised (Singh & Rajaram, 2002).

Group 1 consists out of the family 13H306-4-4 (F_6-5150 – F_6-5153) that showed no positive amplification of rust resistance markers screened for. The phenotype data for the family showed TMR responses. These lines may contain rust resistance genes that was not screened for or minor rust resistance genes with additive effects. There is also a possibility that the lines may obtain non-race specific resistance which is represented by QTL's (quantitative) associated with slow rusting properties (Periyannan *et al.*, 2017). This family's lines would be good candidates to use in future rust resistance breeding.

Group 2, 10 and 11 consisted out of the families 13H317-1-1-1 (F_6-5221 - F_6-5230), 13H273-2-12 (F_6-5689 – F_6-5698) and 13H306-9-5 (F_6-5709 – $F_6$5711). The lines within these families all showed rust resistance responses between 20S and 40S. The molecular profile of these families are similar containing one to three rust resistance genes per line. This indicates that there is stacking of rust resistance genes in the genetic background of these lines that resulted in less susceptibility to leaf rust. Some of the lines also contained the *Lr34* rust resistance gene known for its slow rusting properties, indicating disease presence at low severity levels (Lagudah *et al.*, 2006).

Group 3 and 9 consisted out of the families 13H271-2-1 (F_6-5291 – F_6-5295) and 13H306-8-1 (F_6-5660 – F_6-5663). The lines within these families all showed a leaf rust response of 20S in the field. The molecular profile of the lines within these families showed less rust resistance genes present in the genetic backgrounds compared to group 2, 10 and 11. Only the line F_6-5661 contained the slow rusting *Lr34* gene. The low susceptibility observed could be explained by other leaf rust resistance genes present not screened for or additive gene effects in the genetic background of the lines. The presence of additive genes in plants can be represented with low terminal disease severity under high disease pressure (Singh & Rajaram, 2002).

Group 6, 7 and 8 consisted out of the families 13H099-9 (F_6-5413 – F_6-5416), 13H308-2-5 (F_6-5517 – F_6-5519) and 13H308-2-5 (F_6-5522 – F_6-5524). These families showed a rust phenotype response of 40S. The lines within these family's molecular profiles did not test positive for a lot of resistance genes and also did not contain

stacking of resistance genes. This explained the higher susceptibility responses to leaf rust in the field compared to groups 3 and 9 that only had a 20S responses. The lines that showed 20-40% susceptibility response to leaf rust still gives acceptable levels of resistance. The families' groups 6, 7 and 8 will also be included for future rust resistance breeding purposes.

Group 4 consisted out of the family 11H148-4-4-1-7 (F_6-5296 – F_6-5298). This group's molecular profile doesn't differ much from the groups that showed rust responses of 20S to 40S. This family showed exceptionally high levels of resistance in the field with a R reaction type. This could most likely be explained by the family group containing QTL's linked to rust resistance. A study done by Chen *et al.* (2014) stated that QTL's conferring to resistance can reduce disease expression by having individual small effects and contribute to high levels of resistance in a collective, additive manner. The lines within the family group 11H148-4-4-1-7 will definitely be good candidates to include in future leaf rust resistance breeding.

Velu & Singh (2014) suggested that evaluation of entries be done two to three times in a season between heading and plant maturity. Unfortunately, the rust scoring could not be repeated due to previously mentioned logistical problems and the overlapping of the occurrence of another trial in this study. This trial could not be repeated in the next wheat growing season because of the Covid-19 pandemic travel restrictions and the project's limiting time.

4.4. FHB phenotype trial 2019

The 2019 FHB resistance phenotyping trial consisted out of 29 wheat lines from the 2018 F_6-SUPBL's population and 27 lines from CIMMYT's 21FHBSN015 nursery. Photographs of the inoculated wheat heads were taken on 21 dpi to visualise disease progress (Figure 4.9). A Tinytag data logger was placed in the glasshouse to measure the temperature and humidity every 20 minutes over a period of 6 weeks during the anthesis and kernel development growth stages (Figure 4.10).

The resistant control ‘Sumai3’ had the lowest average disease severity compared to other wheat lines for both spray and point inoculation methods. The average disease severity measured for ‘Sumai3’ showed a low disease progress of 0.00% (14dpi), 10.00% (21dpi) and 21.15% (28 dpi) with the spray inoculation method. The positive control showed a higher average disease severity with the point inoculation method with a disease progress of 5.61% (14dpi), 20.85% (21dpi) and 26.44% (28 dpi). These results can be explained by point inoculation causing more disease because the spore suspension is placed directly into the floret at the fungal penetration site, reducing the chance of disease escape (Schuster and Ellner, 2008; Imathiu *et al.*, 2014). The resistant control, ‘Sumai3’ significantly performed better than the other wheat lines in both the spray and point inoculation methods. The significant lower disease severity observed in ‘Sumai3’ was expected. Two of the major QTL’s linked to FHB resistance, that were screened for in this study, were mapped to ‘Sumai3’ namely *Qfhs.ndsu-3BS* (syn. *Fhb1*) and *Ofhs.ifa-5A* (syn. *Fhb5).* The major QTL’s are linked to FHB resistance Type I (resistance to initial infection) and Type II (resistance to spreading of the disease) (Kosgey, 2019). The susceptible control, 'PAN3471’ showed significant disease with an average disease severity of 79.51% (spray inoculation) and 93.56% (point inoculation) at 28 dpi. This is an indication that the inoculation process was successful and that disease development occurred.

4.4.1. Incidence and severity measurements

The average disease severity for each wheat line at 14, 21 and 28 dpi for both spray and point inoculation methods are displayed in graphs (Figure 4.3-4.8). The lines that performed well compared to ‘Sumai3’ were identified through calculating the LSD on the average disease severity of 28 dpi for every line per inoculation method (Addendum D). The spray inoculation had a calculated average of 50.26, LSD (5%) value of 4.41 and a CV value of 33.19 (Table D1). An average of 73.92, LSD (5%) value of 5.53 and CV value of 28.29 were calculated for the point inoculation method (Table D2). The lines that performed well in the FHB resistance trial were selected for the next season’s FHB phenotyping trial in 2020.

Both the spray and point inoculation methods mimicked FHB field infection. It was decided to only choose one inoculation method for 2020-season FHB phenotyping trial in order to add more replicates to the trial because glasshouse space was a limiting factor. The point inoculation method was the more accurate measurement of disease development because equal amounts of spore suspension were injected into the wheat florets (Imathiu *et al.*, 2014). According to Sydenham (2014) spray inoculation method is used to measure Type I FHB resistance in the field, thus the point inoculation method was chosen to be executed in the next FHB phenotyping glasshouse trial. The point inoculation method's LSD data was used to select the lines that performed well in comparison to 'Sumai3'. The following wheat lines were selected for the FHB phenotyping trial in 2020: F_6-5191 (36.87%), F_6-5200 (34.11%), F_6-5225 (68.52%), F_6-5243 (51.19%), F_6-5297 (68.95%), F_6-5411 (61.7%), F_6-5426 (37.45%), F_6-5429 (45.40%), F_6-5439 (63.9%), F_6-5657 (56.10%), F_6-5661 (56.21%), FHB-6402 (75.56%), FHB-6403 (52.36%), FHB-6407 (71.10%), FHB-6408 (50.00%), FHB-6409 (75.81%), FHB-6410 (70.96%), FHB-6411 (61.83%), FHB-6414 (55.77%), FHB-6415 (52.27%), FHB-6426 (68.06%), FHB-6432 (78.02%), FHB-6434 (62.95%) and FHB-6435 (47.62%).

The data analysis calculated a R^2 of 0.7083, showing a relatively good model fit. The CV was calculated as 51.82% which is high and indicates less reliable data. This could be explained by extremely high temperature conditions the plants experienced during anthesis in the glasshouse. The glasshouse also experienced temperatures that fluctuated between 11.14°C and 55.56°C which is far beyond the optimal temperature for disease development. Due to these extreme temperatures some of the plants died because of drought stress which resulted in loss of data and replicates of some of the wheat lines. The heat in the glasshouse also resulted in some of the plants that matured too fast and started drying off between 21 and 28 dpi resulting in wheat head discolouration of yellow-whitish colour. In a study done by Acevedo *et al*. (2002) stated that spring wheat plants matures faster when exposed to environmental stress such as heat. The fast maturing plants could not be used for further evaluation because no distinction could be made between head discolouration of FHB disease presence or the plant drying off because of maturing. This also resulted in loss of data points.

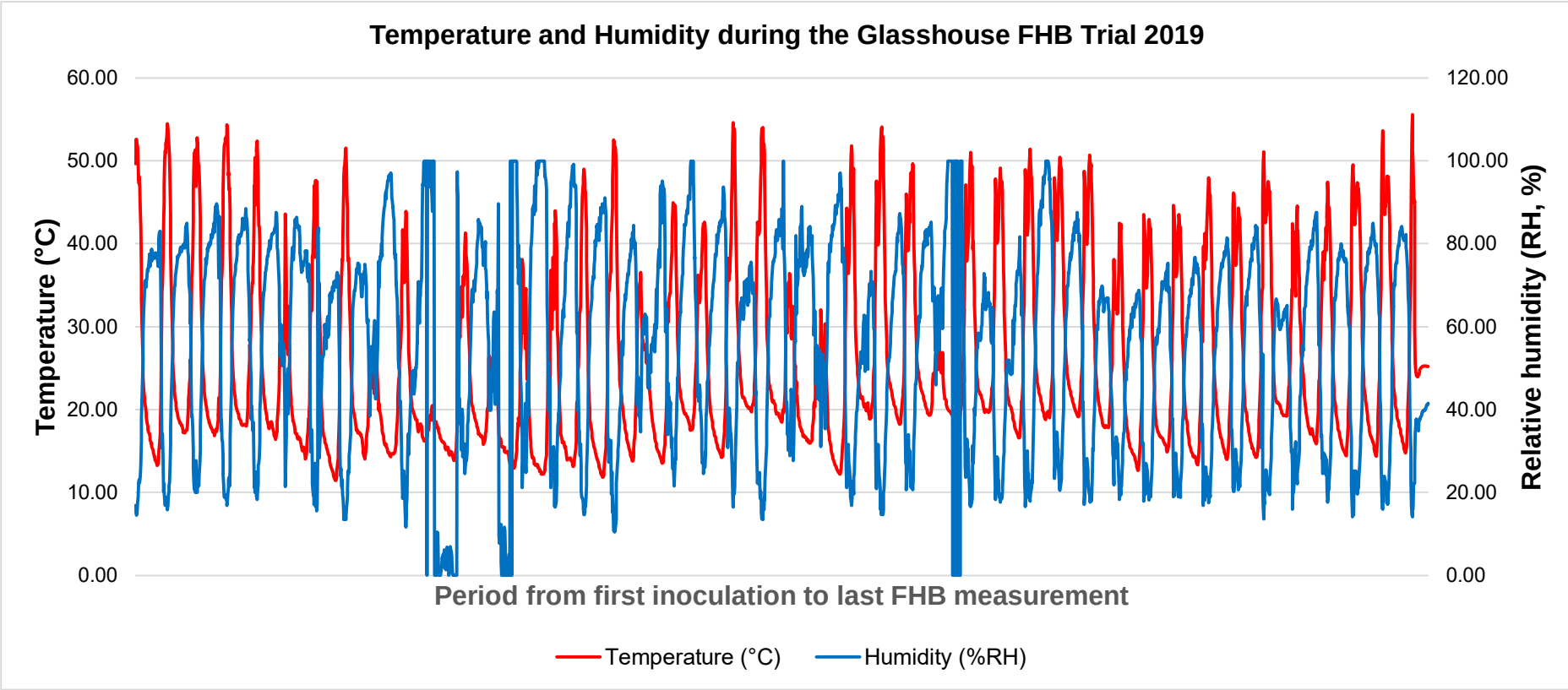

Figure 4.2. Temperature and relative humidity measured in FHB phenotyping trial 2019.

The temperature varied between 11.41°C and 55.56°C with an average of 25.65°C. The average relative humidity measured was 55.14% and fluctuated between 0.00% and 100.00%. When the data logger becomes in contact with water for instance during irrigation it can give a reading of 0.00% relative humidity.

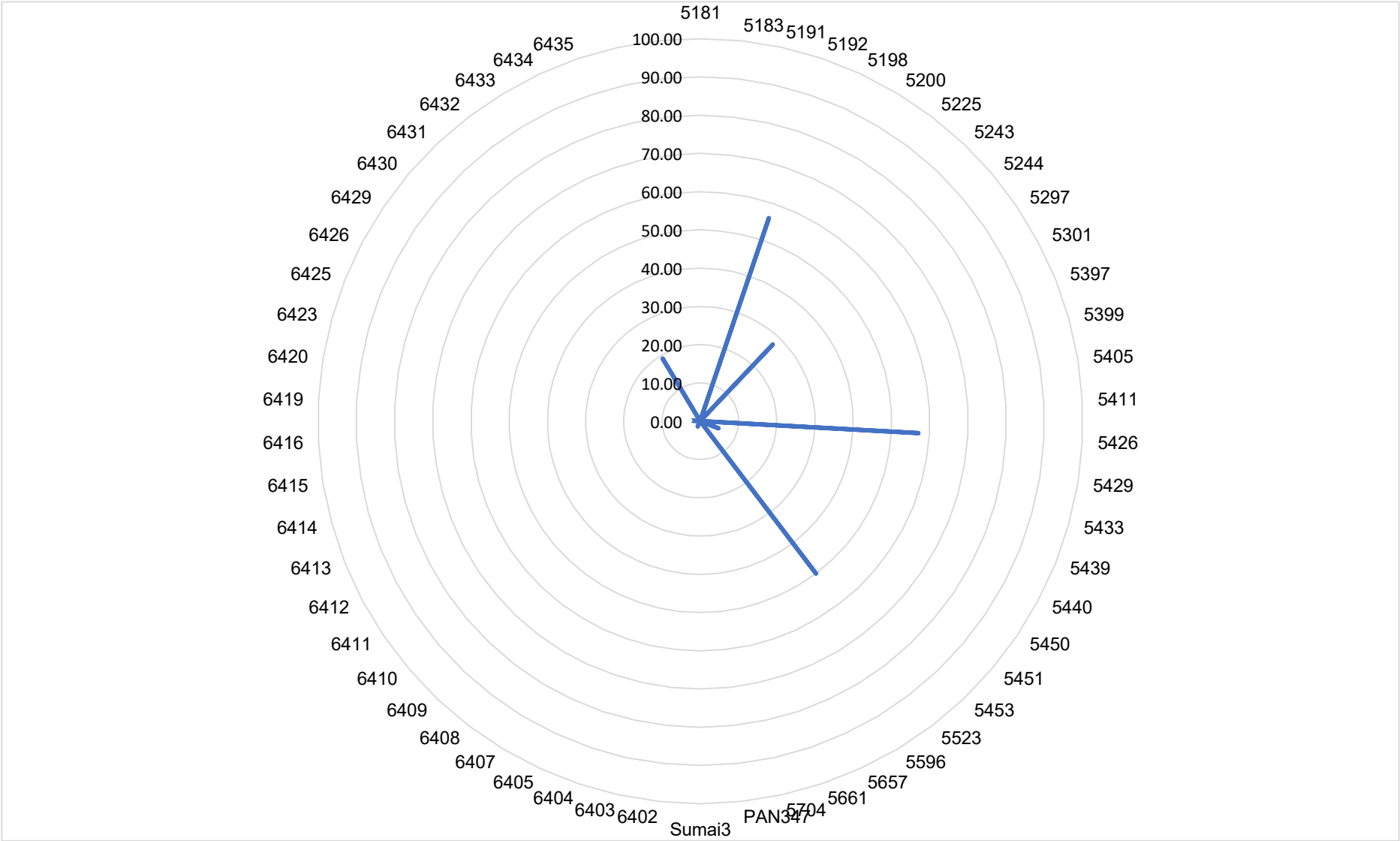

Figure 4.3. The average disease severity of the spray inoculation method at 14 dpi in FHB trial 2019.

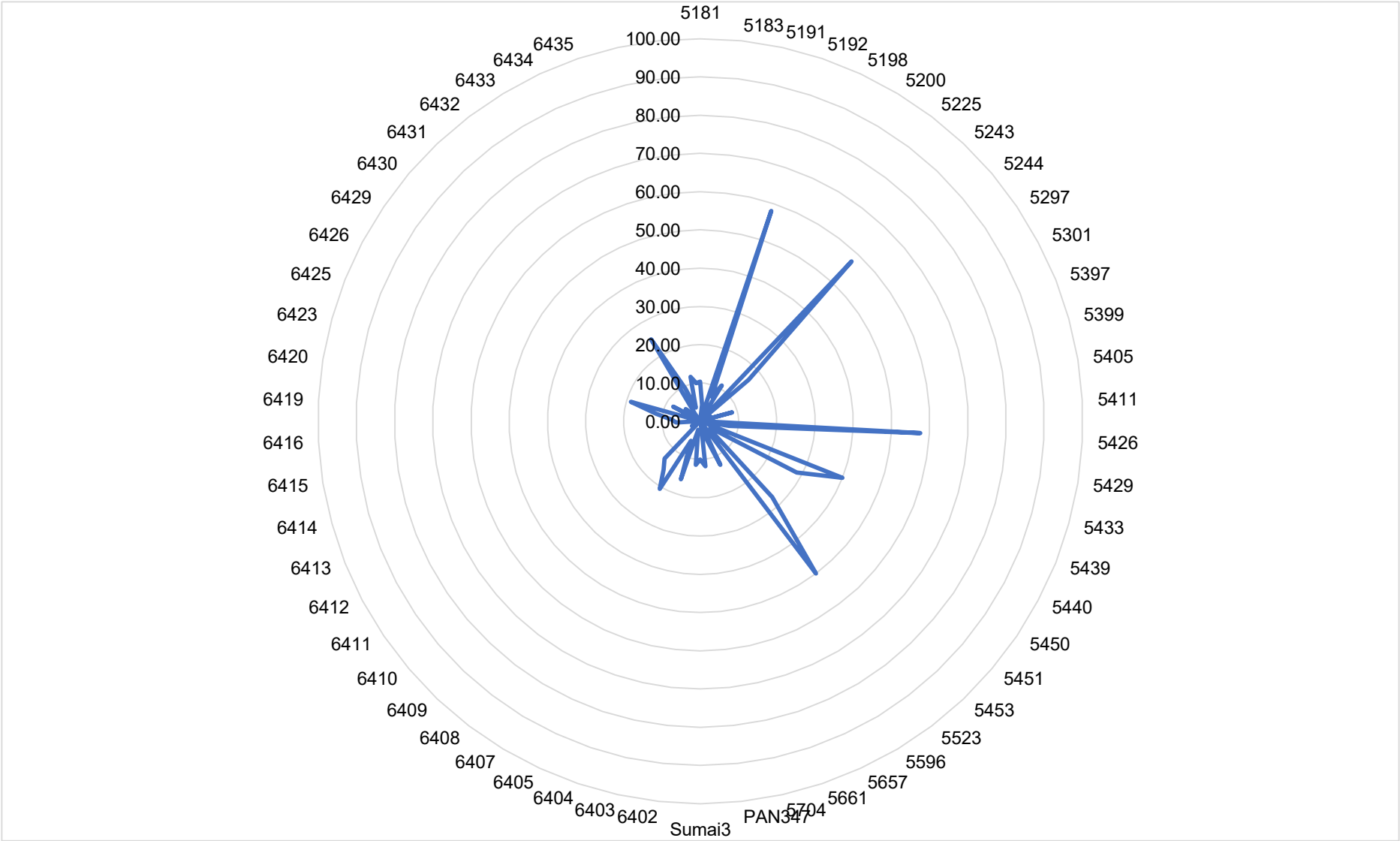

Figure 4.4. The average disease severity of the spray inoculation method at 21 dpi in FHB trial 2019.

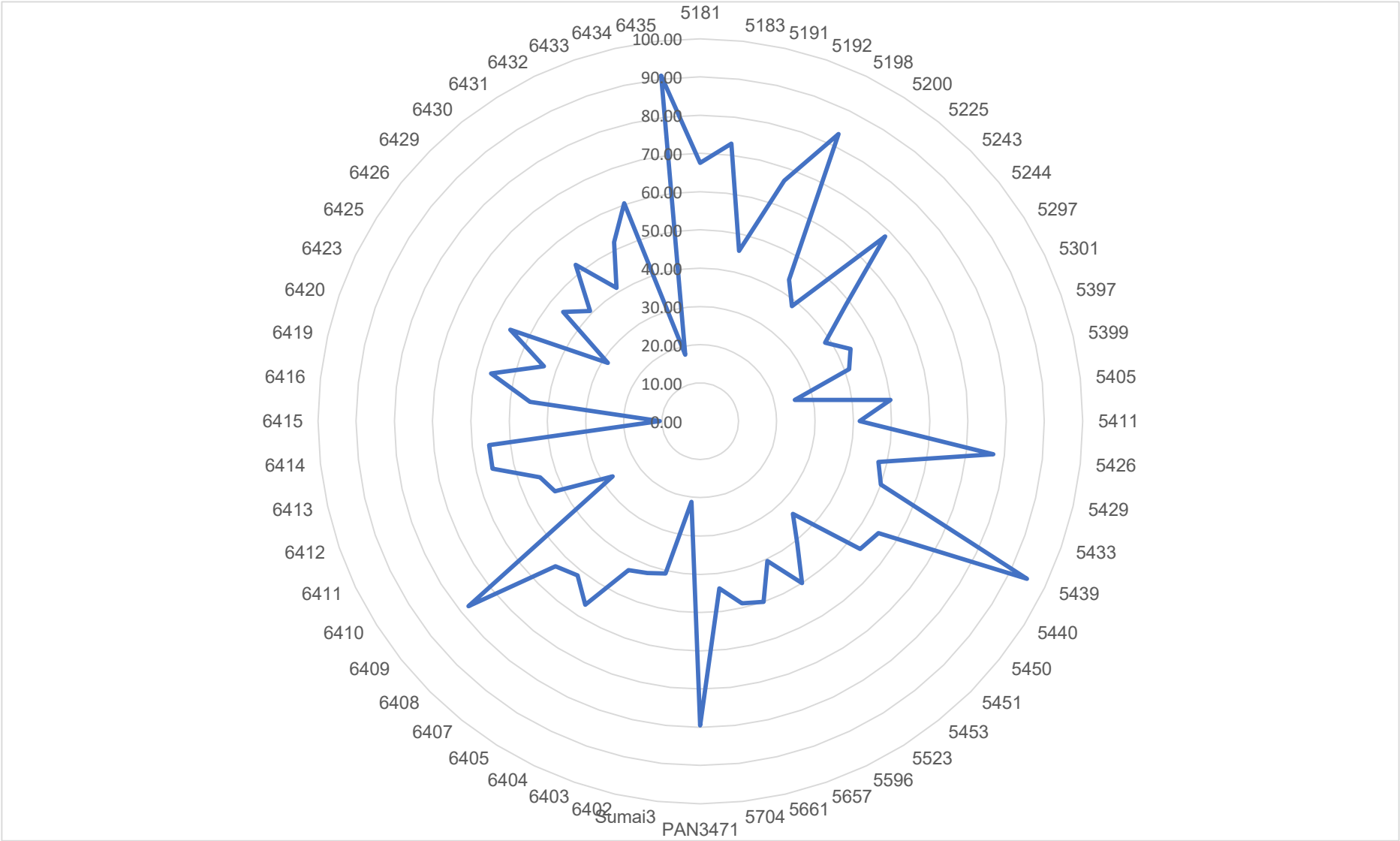

Figure 4.5. The average disease severity of the spray inoculation method at 28 dpi in FHB trial 2019.

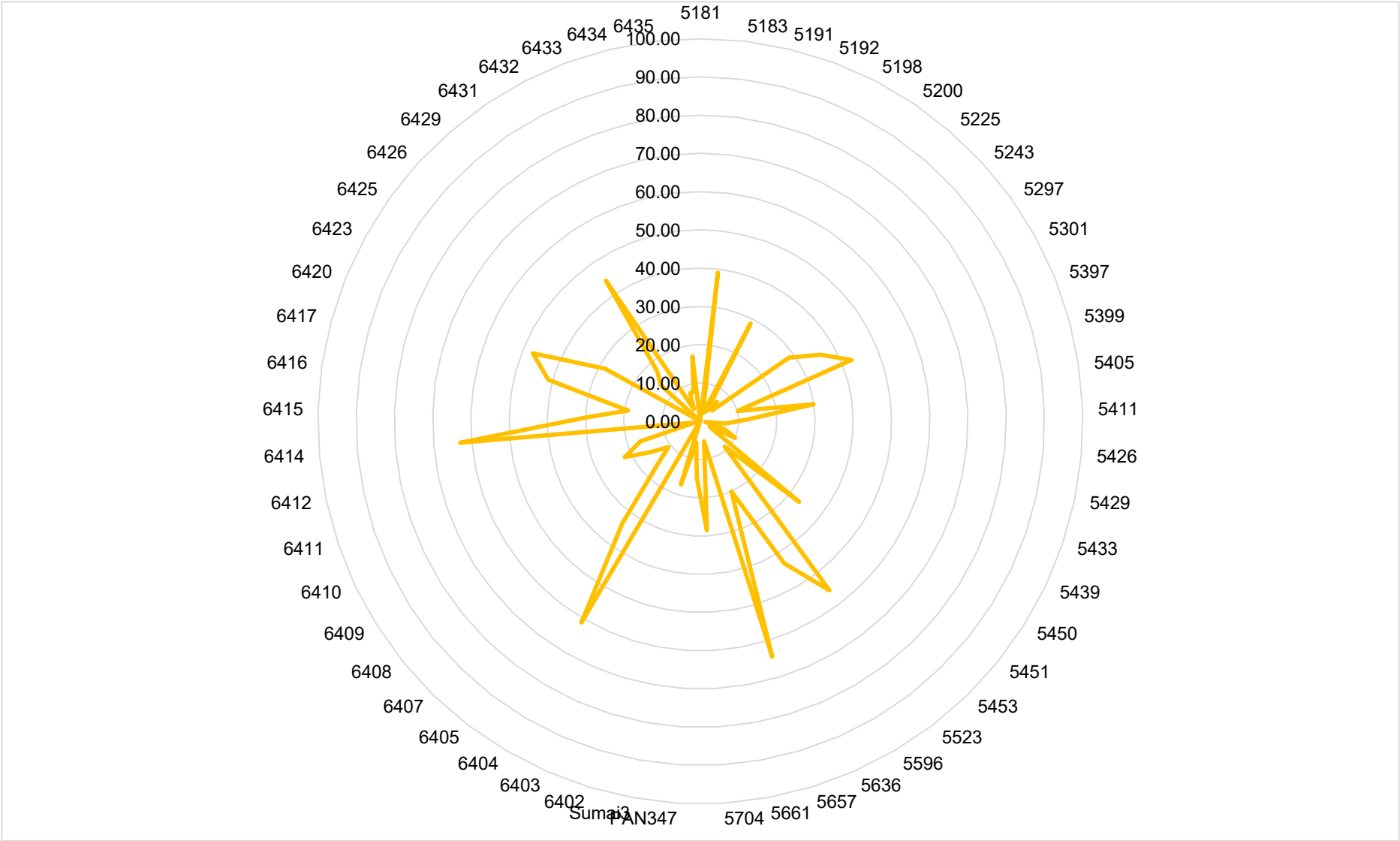

Figure 4.6. The average disease severity of the point inoculation method at 14 dpi in FHB trial 2019.

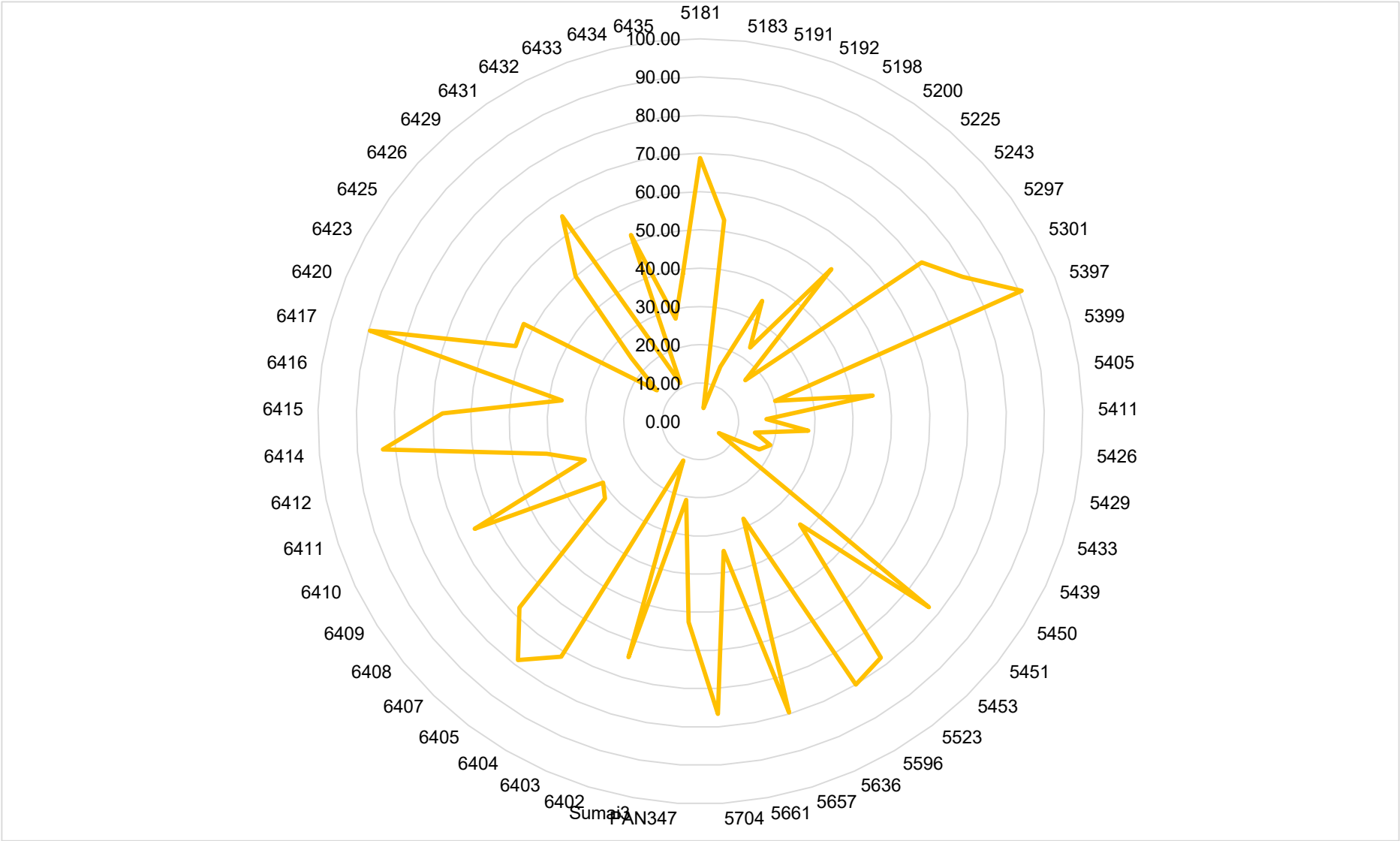

Figure 4.7. The average disease severity of the point inoculation method at 21 dpi in FHB trial 2019.

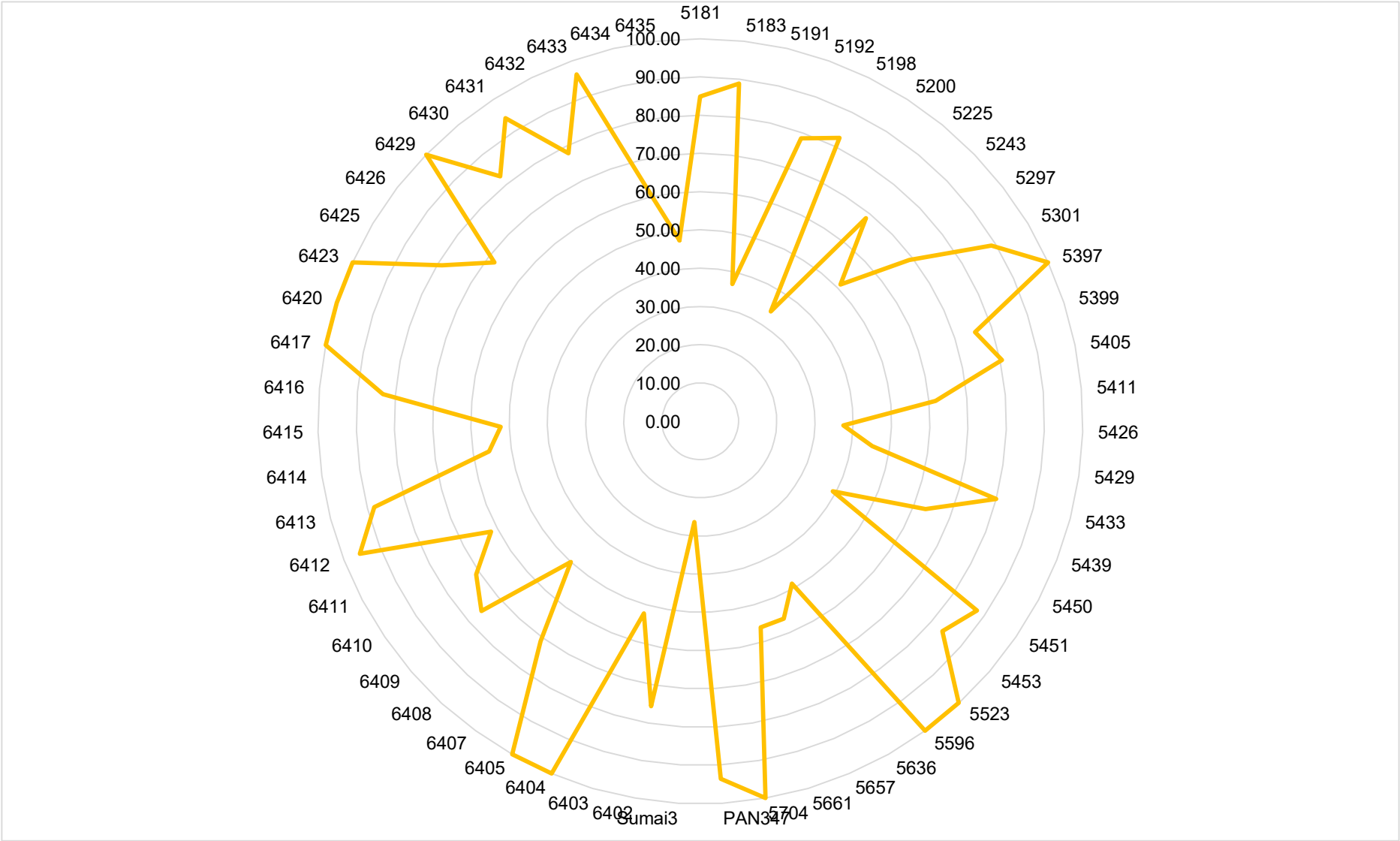

Figure 4.8. The average disease severity of the point inoculation method at 28 dpi in FHB trial 2019.

Figure 4.9. Photos of inoculated wheat ears 21 days post inoculation displaying disease progress of FHB.

a) 13%, b) 38%, c) 63%, d) 71%, e) 88% and f) 100%.

4.5. FHB phenotype trial 2020

The FHB phenotyping trial of 2020 consisted out of 13 wheat lines from the 2018 F_6-SUPBL's population and 13 lines from CYMMIT's 21FHBSN015 nursery. The graph (Figure 4.10) displays the temperature and relative humidity over a period of 11 weeks.

4.5.1. Incidence and severity measurements

The average disease severity of the lines was calculated over the three replicates on 14, 21 and 28 dpi and are displayed in graphs (Figure 4.11-4.13). Photographs were taken to visualise disease progress at 21 dpi (Figure 4.14). The resistant control, 'Sumai3', showed the lowest average disease severity of 1.68% at 28 dpi over three replicates (Table 4.6). The negative control, 'PAN4371', showed an average disease severity development of 11.15% (14 dpi), 14.06% (21 dpi) and 16.65% (28 dpi). This was an indication that the FHB disease was present in the glasshouse.

The data analysis calculated a R^2 value of 0.5941. The R^2 value is very low indicating that the estimated data and the actual data did not correlate well. The data analysis also showed a high CV of 114.6% indicating that the integrity of the data is questionable. These results could be explained by the lack of infection because of unfavourable environmental conditions experienced in the glasshouse. The glasshouse experienced temperatures that fluctuated between 6.8°C and 56.8°C while disease development is optimal at 25°C for FHB fungal growth (Leplat *et al*., 2013). The experienced high and low temperatures would hinder optimal disease development.

There was also powdery mildew disease (a common problem) in the glasshouse. Five wheat lines (F_6-5411, F_6-5439, FHB-6403, FHB-6408 and FHB-6410) were highly susceptible to the disease and were removed from the trial resulting in the loss of 15 data points. The glasshouse could not be sprayed for powdery mildew disease during the FHB inoculations because the effect of fungicides on the FHB disease infection and development is unknown. Research on the interaction between FHB and powdery mildew diseases on wheat is limited. In a study done by Li-qin *et*

al., (2018) reported that the expression of a *TaSSI2* gene in wheat resulted in FHB and powdery mildew resistance. The gene has the ability to positively regulate the abscisic acid (ABA) and salicylic acid (SA) pathways implying that *TaSSI2* is involved in multiple biological functions. There is a possibility that the powdery mildew infection could already activated an immune response influencing the FHB infection establishment within the wheat plant. This could add to the inaccuracy of the data explained by the high CV value. Unfortunately, this experiment could not be repeated to achieve more accurate and reliable results because of the time limiting factor of the project.

Table 4.6. The average Fusarium head blight disease severity of each wheat line replicate at 28 dpi.

Wheat Line	Average disease severity per line (%)				Average disease severity per line (%)
	Rep 1	Rep 2	Rep 3	Water rep	
F_6-5191	3.94	12.65	6.71	0.00	7.77
F_6-5200	5.81	49.42	4.27	0.00	19.83
F_6-5225	1.19	0.00	12.00	0.00	4.40
F_6-5243	0.00	10.22	0.00	0.00	3.41
F_6-5297	1.19	0.78	2.78	0.00	1.58
F_6-5426	1.32	11.07	6.57	0.00	6.32
F_6-5429	0.00	4.03	2.73	0.00	2.25
F_6-5450	0.00	3.18	2.08	0.00	1.75
F_6-5636	3.89	0.00	2.09	0.00	1.99
F_6-5657	4.50	4.16	0.00	0.00	2.89
F_6-5661	5.00	16.53	3.69	0.00	8.41
FHB-6402	1.75	2.66	2.86	0.00	2.42
FHB-6407	5.36	55.88	26.39	0.00	29.21
FHB-6409	53.09	10.90	32.48	0.00	32.16
FHB-6411	17.29	0.98	0.75	0.00	6.34
FHB-6414	0.83	22.85	10.46	0.00	11.38
FHB-6415	17.64	5.21	6.52	0.00	9.79
FHB-6426	0.00	4.13	0.00	0.00	1.38
FHB-6432	16.84	2.15	2.78	0.00	7.26
FHB-6434	1.67	27.22	8.38	0.00	12.42
Sumai3	0.00	0.00	5.04	0.00	1.68
PAN4371	11.15	14.06	16.65	0.00	13.95

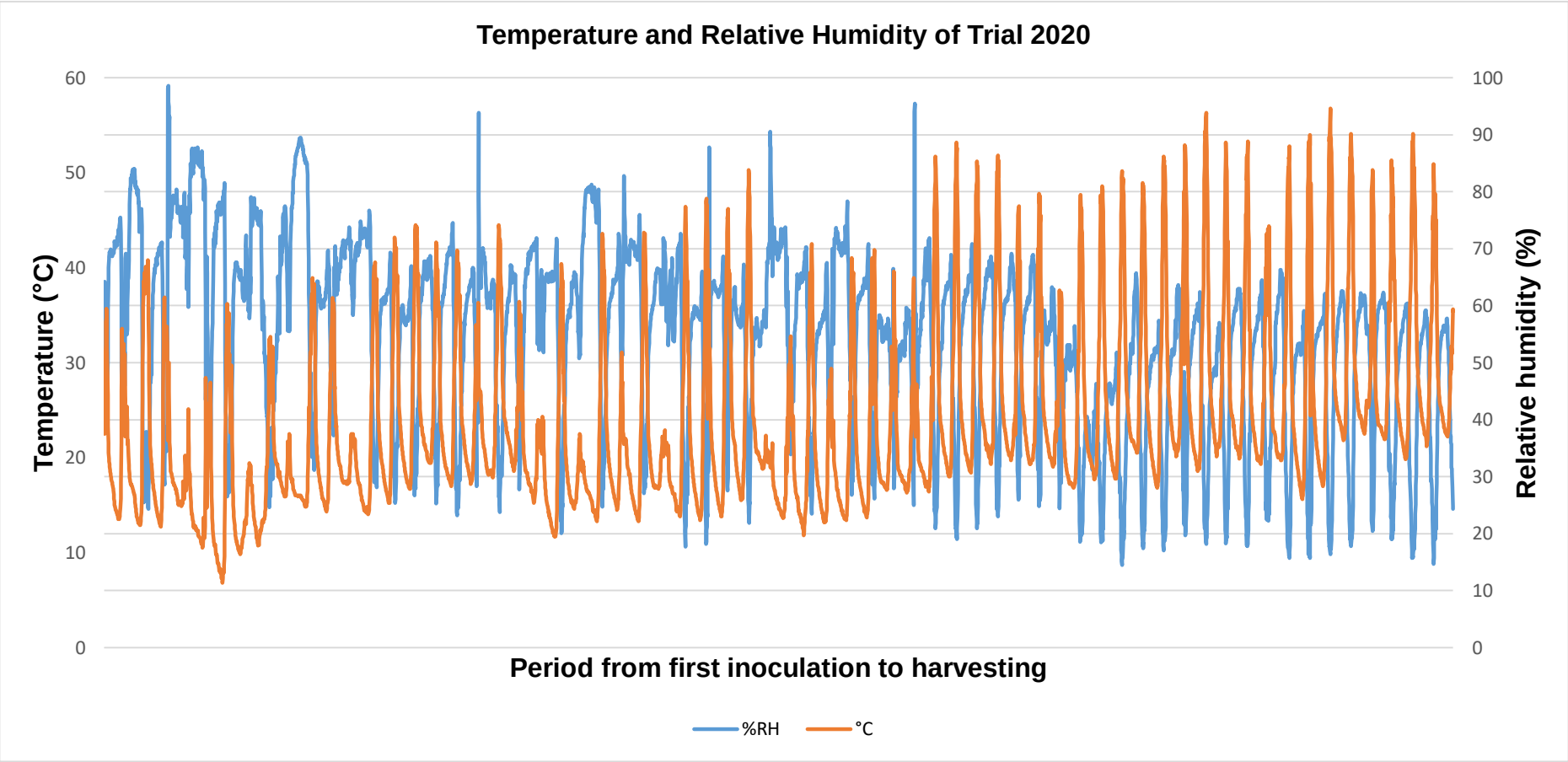

Figure 4.10. The temperature and relative humidity measured in the FHB phenotyping trial 2020.

The relative humidity varied from 14.5% to 98.6% with an average of 54.2%. The average temperature measured was 23.9°C and fluctuated between 6.8°C and 56.8°C.

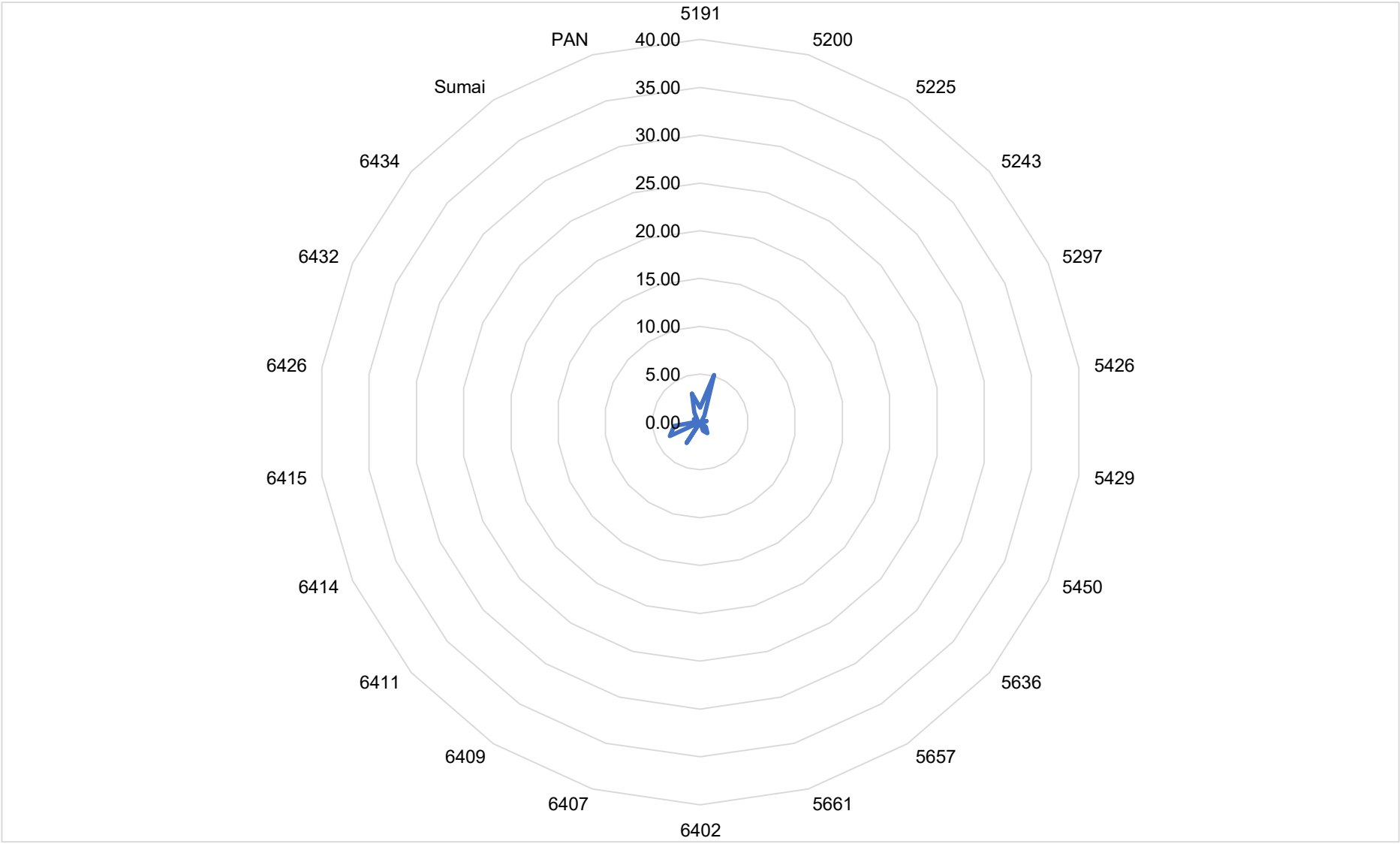

Figure 4.11. The average disease severity of the FHB trial 2020 at 14 dpi.

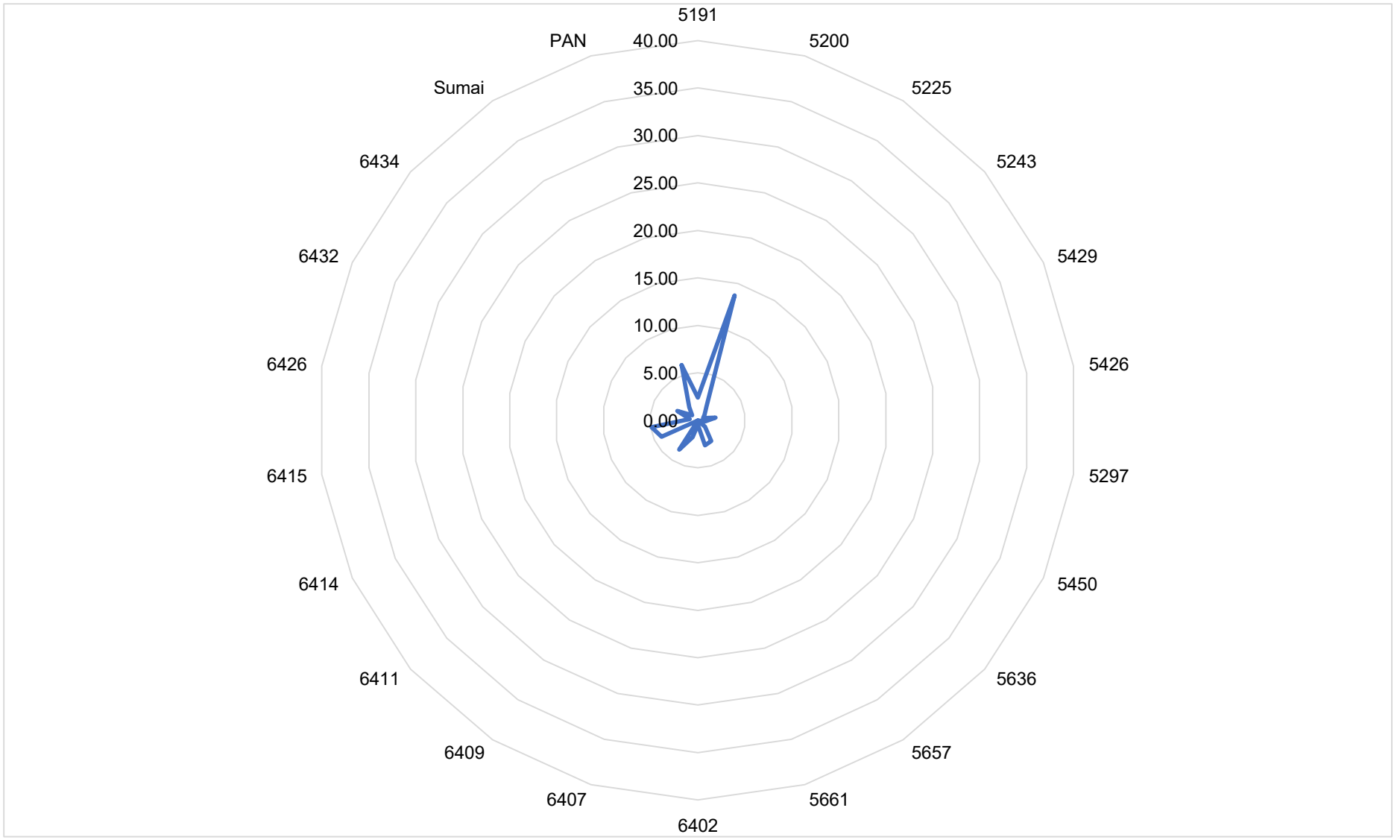

Figure 4.12. The average disease severity of the FHB trial 2020 at 21 dpi.

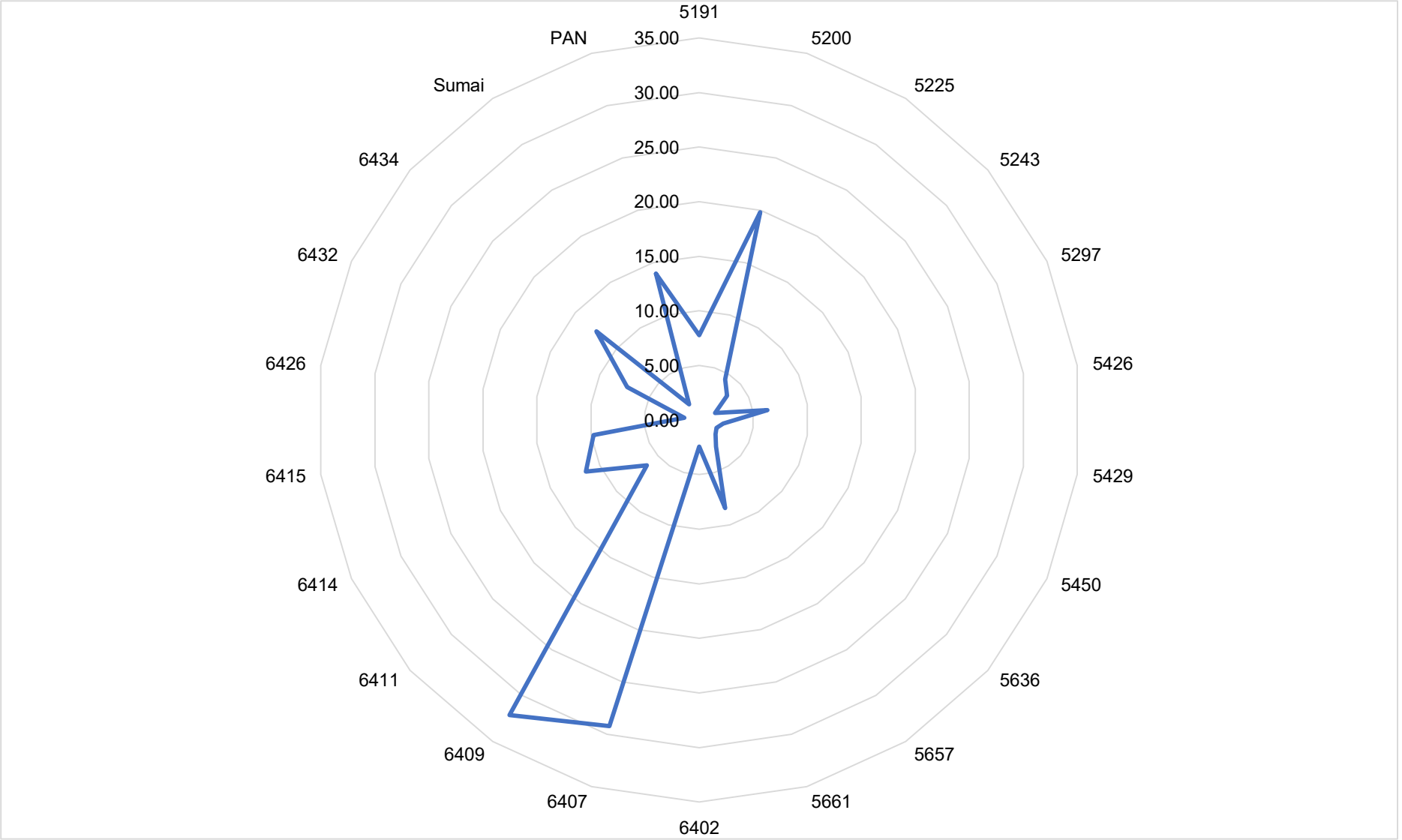

Figure 4.13. The average disease severity of the FHB trial 2020 at 28 dpi.

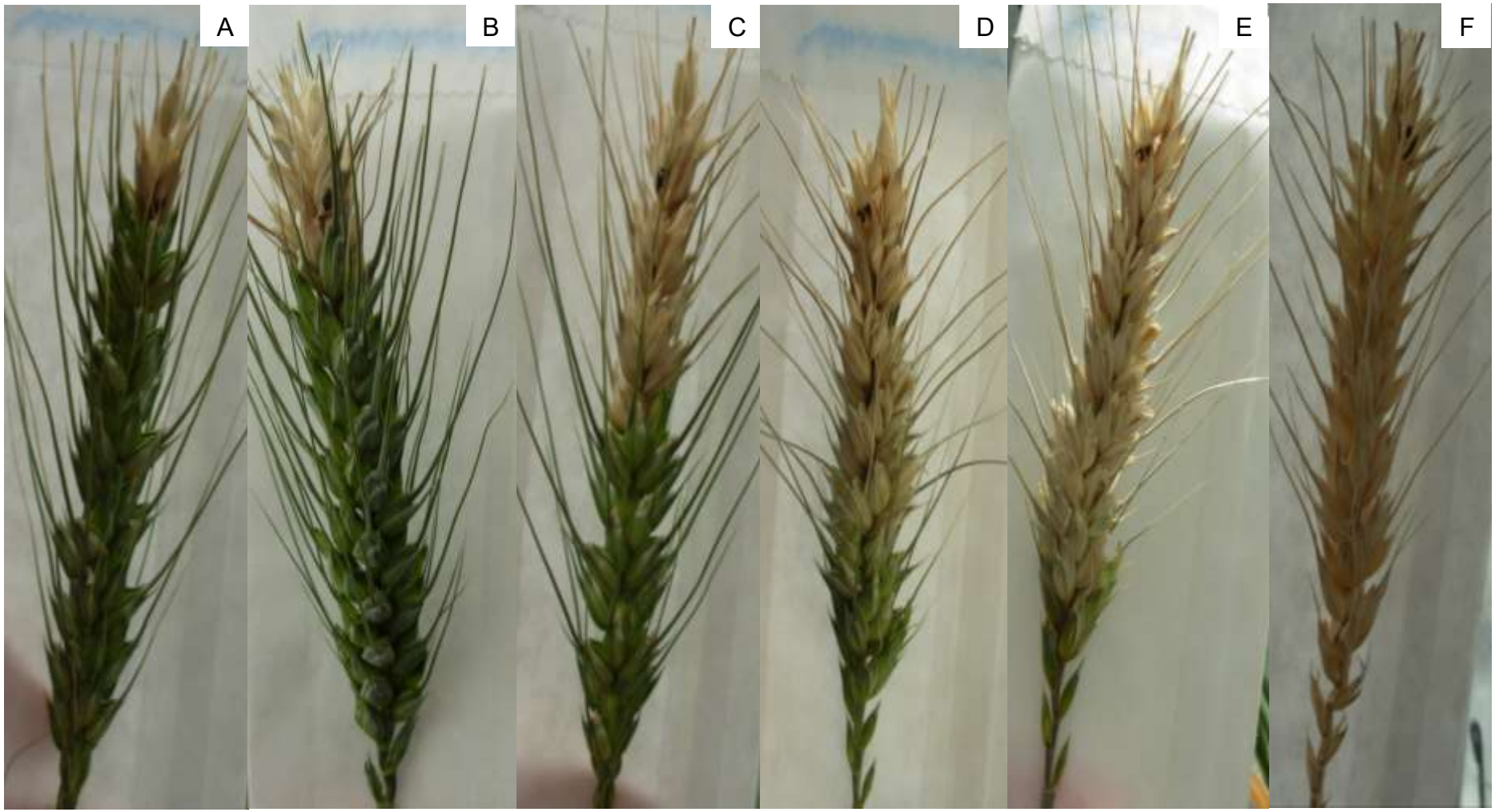

Figure 4.14. Photos of inoculated wheat ears 21 days post inoculation displaying disease progress of FHB.

a) 22%, b) 30%, c) 50%, d) 66%, e) 88% and f) 100%.

4.5.2. Mycotoxin analyses

Mycotoxin contamination of grains produced by FHB-causal pathogens is a threat to the wheat industry's products before and after processing. A mycotoxin analysis was performed on the wheat lines to screen for FHB Type III resistance in the 2020 FHB phenotyping trial. The standard curves for DON, NIV and ZEA had the following coefficient of determination (R^2): 0.999679, 0.999924 and 0.998431.

The LC-MS/MS technique can only measure accurate NIV concentrations at 0.032 ppb. The *F. graminearum* isolates used during the trials' inoculations were not NIV-producing isolates. It can be concluded that grain samples from this trial contained no NIV contamination. The contamination of NIV via *F. graminerum* species in South African grains occurs less in comparison to DON contamination according to Beukes *et al.* (2018). The LC-MS/MS technique can only measure accurate ZEA concentrations at 0.064 ppb. The mycotoxin data revealed that the grain samples did not show ZEA contamination above 0.064 ppb. These results could be explained that ZEA contaminations in the field is usually low but can increase in storage conditions reported by Agriopoulou *et al.* (2020). Zearalenone contamination in stored grains can increase with moisture levels between 30-40% and at temperatures of 25°C (Mylona *et al.*, 2012; Agriopoulou *et al*., 2020). The LC-MS/MS technique can only measure accurate DON concentrations at 0.032 ppb. The data received from CAF showed that most of the samples' DON contamination were below 0.032 ppb. The FHB phenotype trial 2020 data showed disease infection and low mycotoxin contamination in grains. This is supported by a study done by Góral *et al.* (2019) that reported DON contamination and visual wheat head infection had no correlation. The low DON contamination levels of the samples can be explained by the unfavourable environmental conditions experienced. The glasshouse temperature fluctuated between 6.8°C to 56.8°C which is not optimal for fungal growth. This resulted in less fungal growth leading to less production of DON in grains. The resistant control, 'Sumai3' showed a DON contamination of lower than 0.032 ppb. This was expected because 'Sumai3' contains the major QTL *Fhb3* (syn. *Fhb7AC*) that is linked to Type III resistance (Kosgey, 2019). The susceptible control 'PAN 4731' had a DON contamination of 0.310 ppb. Wheat lines with grain samples that tested above 0.032 ppb for the mycotoxin DON are listed in Table 4.7.

Table 4.7. The wheat lines in the FHB phenotyping trial 2020 that were contaminated with mycotoxins.

Wheat line	Replicate	Mycotoxin	Concentration (ppb)
F_6-5657	1	DON	0.035
F_6-5661	1	DON	0.0334
FHB-6415	1	DON	0.032
F_6-5657	2	DON	0.152
FHB-6409	2	DON	0.060
FHB-6415	2	DON	1.212
FHB-6426	2	DON	0.017
FHB-6432	2	DON	0.015
F_6-5191	3	DON	0.313
F_6-5200	3	DON	0.0248
F_6-5225	3	DON	2.378
F_6-5243	3	DON	0.033
F_6-5297	3	DON	0.077
F_6-5426	3	DON	0.370
F_6-5429	3	DON	0.123
FHB-6414	3	DON	1.370
FHB-6415	3	DON	0.404

According to Beukes *et al.* (2018) the mycotoxin DON level in South African grains are limited by 2000 µg kg^{-1} (ppb) before processing. The samples that tested above 0.032 ppb for DON contamination are well under the regulated limits. The wheat line F_6-5657 had a DON contamination of 0.035pp (rep 1) and 0.152 (rep 2), while FHB-6415 had a DON contamination in rep 1, 2 and 3 of 0.032 ppb, 0.152 ppb and 0.404 ppb. From a breeder's perspective these two lines will still be excluded from future FHB resistance breeding. The sample F_6-5225 showed a DON contamination of 2.378 ppb only in replicate three. According to a study done by Smaoui *et al.* (2020) the LC-MS/MS procedure is a very selective technique but can lead to false positive results. This is because the signal can be lost in challenging samples or be overestimated. The LC-MS/MS can also not detect trace levels of some analytes when there was compromises related to preparation of samples despite being described as a highly sensitive analytical technique. Smaoui *et al.* (2020) also reports that the mycotoxin contamination process can be seen as "spot processes" because fungal growth and distribution are limited to certain locations.

Chapter 5: Conclusions

The donor populations (SUBPL 2018 F_6 germplasm and 21FHBSN015 CIMMYT Nursery) were successfully molecularly characterised for the presence of rust resistance genes (*Lr34, Lr37*, *Sr2, Sr24, Sr26* and *Sr31*) and markers linked to FHB resistance (*Qhfs.ifa-5A, 7AQTL* and *Qfhs.ndsu-3BS*). The molecular data generated contributed to the selection of donor lines containing desirable traits that were included as parents in crossing cycles. The segregating male sterile populations (F_0 and F_1) were also successfully characterised for the presence of leaf and stem rust resistance genes *Lr34* and *Sr2*. The F_1 male population could not be successful screened for the presence of the male sterility gene, *Ms3*. The marker could not correctly discriminate between the presence or absence of the *Ms3* gene in the male sterile population. The study relied on visible phenotypic traits linked to sterility to identify sterile tillers.

Successful cross-pollination was accomplished in MS-MARS cycle 1 and 2. The MS-MARS cycle 1 and cycle 2 had a total average cross pollination of 42.93% and 43.64%. The cross pollination percentage could be increased by keeping glasshouse temperatures between 16°C and 25°C during anthesis to prevent the *Ms3* gene of becoming unstable. This would resulted in less self-pollination and increase the number of sterile tillers included in crossing events. In both MS-MARS cycle 1 and 2 the male sterile population showed a 1:1 segregating ratio. This shows the dominance of the *Ms3* gene in the female populations.

The selected wheat lines with desirable rust resistance genes were evaluated during a rust phenotyping field trial. The performance of the lines under a field rust epidemic could be compared to the molecular data generated. The comparison helped to identify potential parents to include in future rust resistance breeding. The reliability of the results could be improved by adding data of two to three rust scoring events during the wheat growing season. To further complete the rust phenotyping data, stem rust scorings should be included. The rust phenotyping field trial should be performed over three wheat growing seasons to reduce the genotype x environment interaction.

Successful inoculations were performed in the FHB phenotyping glasshouse trials by using the pipette point inoculation method. This could be validated by the visual symptoms appearing on wheat heads. Wheat lines could be scored for Type II resistance at 14, 21 and 28 dpi. Lines that performed well compared to the positive control 'Sumai 3' were selected to be included as parents in the next crossing cycle. Successful mycotoxin analysis could not be performed because of unreliable data. The FHB phenotyping trials could be improved by keeping the glasshouse temperatures at an optimum range between 20°C and 25°C to increase disease development and mycotoxin production. FHB phenotyping trials should be performed on the same wheat lines over two or more seasons.

The aim of this study was achieved by identifying four wheat lines (F_6-5450, F_6-5636, FHB-6411 and FHB-6434) that could be potential parents to include in the future MS-MARS pre-breeding program. These lines were identified through a collection of different MAS and phenotyping data. The lines F_6-5450 and F_6-5636 showed 40S and 20S responses to field leaf rust infection. The lines showed positive amplification for six markers linked to baking quality and yield traits. Both the lines had a low average disease severity in the FHB trials and the LC-MS/MS technique did not measure DON contamination levels above 0.032 ppb. The FHB-6411 and FHB-6434 also showed low average disease severity (spray and point inoculation) in both FHB trials and no DON contamination levels above 0.032 ppb. The FHB-6434 line showed positive amplification for four baking quality and yield markers. The FHB-6411 line tested positive for the *Lr37* gene cluster and six markers linked to yield and baking quality traits. Both the FHB-6411 and FHB-6434 wheat lines were included in the 21FHBSN015 CIMMYT Nursery from Mexico, bringing in new genetic material that can benefit the SU-PBL's base population.

Future work should aim at measuring Type I, Type II and Type III FHB resistance over two to three wheat growing seasons. This will broaden the FHB resistance data of wheat lines which will improve the selection of parents in MS-MARS crossing cycles. Increasing the number of replicates in FHB phenotyping trials will result in more reliable data, keeping in mind the amount of labour needs during the inoculation process. Future studies should also attempt to store harvested seeds of inoculated wheat heads for longer periods to measure resistance to accumulation of the ZEA mycotoxin.

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Addendum A

Table A.1.The rust resistance molecular data and rust resistance phenotyping data of the donor material.

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5140	0	0	0	0			0
F_6-5141	0	0	0	0			0
F_6-5142	0	0	0	0			0
F_6-5143	0	0	0	0			0
F_6-5144	0	0	0	0			0
F_6-5145	0	0	0	0			0
F_6-5146	0	0	0	0			0
F_6-5147	0	0	0	0			0
F_6-5148	0	0	0	0			0
F_6-5149	0	0	0	0			0
F_6-5150	0	1	0	0			1
F_6-5151	0	0	0	0	0	0	0
F_6-5152	0	0	0	0	0	0	0
F_6-5153	1	0	0	0	0	0	1
F_6-5154	0	0	0	0	0	0	0
F_6-5155	0	0	0	0			0
F_6-5156	0	0	0	0			0
F_6-5157	1	0	0	0			1
F_6-5158	1	0	0	0			1
F_6-5159	1	0	0	0			1
F_6-5160	0	0	0	0			0
F_6-5161	0	0	0	0			0
F_6-5162	0	0	0	0			0
F_6-5163	0	0	0	0			0

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5164	0	0	0	0			0
F_6-5165	1	0	0	0			1
F_6-5166	0	0	0	0			0
F_6-5167	1	0	0	0			1
F_6-5168	0	0	0	0			0
F_6-5169	0	0	0	0			0
F_6-5170	0	0	0	0			0
F_6-5171	0	0	0	0			0
F_6-5173	0	0	0	0	0	0	0
F_6-5174	0	1	1	0			2
F_6-5176	0	1	0	0	0	0	1
F_6-5177	0	0	0	0			0
F_6-5178	1	0	0	1			2
F_6-5179	0	0	0	0	0	0	0
F_6-5181	0	0	0	0	0	0	0
F_6-5182	0	1	1	0	0	0	2
F_6-5183	0	0	0	0	0	0	0
F_6-5184	0	1	1	0	0	0	2
F_6-5185	0	1	0	0	0	1	2
F_6-5186	0	1	0	0	0	1	2
F_6-5187	0	0	0	0	0	1	1
F_6-5188	0	0	0	0	0	1	1
F_6-5189	0	0	0	0	0	1	1
F_6-5190	0	0	0	0	0	0	0
F_6-5191	0	0	0	0	0	0	0
F_6-5192	0	0	0	0	0	0	0
F_6-5193	0	0	0	0	0	0	0

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5194	0	0	0	0	0	0	0
F_6-5195	0	0	0	0	0	0	0
F_6-5196	0	0	0	0			0
F_6-5197	0	1	0	0	0	0	1
F_6-5198	0	1	0	0	0	0	1
F_6-5199	0	0	0	0	0	0	0
F_6-5200	0	0	0	0	0	0	0
F_6-5201	1	0	0	1	0	0	2
F_6-5202	1	0	0	1	0	0	2
F_6-5203	0	0	0	0	0	1	1
F_6-5204	1	0	0	1	0	0	2
F_6-5205	0	0	0	1	0	0	1
F_6-5206	1	0	0	1			2
F_6-5207	0	0	0	1	0	0	1
F_6-5208	1	1	0	1	0	1	4
F_6-5209	1	1	0	1	0	0	3
F_6-5210	1	0	0	1	0	0	2
F_6-5211	0	0	0	0	0	1	1
F_6-5212	0	0	0	0	0	1	1
F_6-5213	0	0	0	0	0	0	0
F_6-5214	0	1	1	0	0	1	3
F_6-5215	0	0	0	0	0	1	1
F_6-5216	0	1	1	0	0	1	3
F_6-5217	0	0	0	0	0	1	1
F_6-5218	0	0	0	0	0	1	1
F_6-5219	0	1	0	0	0	1	2
F_6-5220	0	0	0	0			0

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5221	0	0	0	0	0	1	1
F_6-5222	0	0	0	0	0	1	1
F_6-5223	0	1	0	0	0	1	2
F_6-5224	0	0	0	0	0	1	1
F_6-5225	0	1	0	0	0	0	1
F_6-5226	0	0	0	0	0	1	1
F_6-5227	0	0	0	0			0
F_6-5228	0	0	0	0			0
F_6-5229	0	0	0	0	0	1	1
F_6-5230	0	0	0	0	0	1	1
F_6-5231	0	1	0	0			1
F_6-5232	0	1	0	0			1
F_6-5233	0	1	0	0	0	1	2
F_6-5234	0	0	0	0	0	1	1
F_6-5235	0	0	0	0	0	0	0
F_6-5236	0	0	0	0	0	1	1
F_6-5237	0	0	0	0	0	1	1
F_6-5238	0	0	0	0	0	0	0
F_6-5239	0	0	0	0	0	1	1
F_6-5240	0	0	0	0	0	1	1
F_6-5241	1	0	0	1	0	1	3
F_6-5243	0	0	0	0	0	0	0
F_6-5244	0	0	0	1	0	0	1
F_6-5245	0	0	0	1			1
F_6-5246	0	0	0	0			0
F_6-5247	1	0	0	1			2
F_6-5248	1	0	0	1			2

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5249	1	0	0	1			2
F_6-5250	1	0	0	1			2
F_6-5252	1	1	0	0			2
F_6-5253	0	0	0	0			0
F_6-5254	1	1	0	0			2
F_6-5255	1	1	0	0			2
F_6-5256	1	1	0	0			2
F_6-5257	1	1	0	0			2
F_6-5258	1	1	0	0			2
F_6-5259	1	1	0	0			2
F_6-5260	1	1	0	0			2
F_6-5261	0	0	0	0	0	0	0
F_6-5262	0	0	0	0			0
F_6-5263	0	0	0	0			0
F_6-5264	0	0	0	0			0
F_6-5265	0	0	0	0	0	1	1
F_6-5266	0	0	0	0			0
F_6-5267	0	0	0	0			0
F_6-5268	0	0	0	0			0
F_6-5269	0	0	0	0			0
F_6-5270	1	1	0	0			2
F_6-5271	0	0	0	0	0	1	1
F_6-5272	0	1	0	0			1
F_6-5273	0	1	0	0	0	1	2
F_6-5274	0	1	0	0	0	0	1
F_6-5275	0	1	0	0	0	1	2
F_6-5276	0	0	0	0	0	1	1

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5277	0	1	0	0	0	0	1
F_6-5278	0	0	0	0	0	1	1
F_6-5279	0	1	0	0	0	1	2
F_6-5280	0	0	0	0	0	0	0
F_6-5281	0	0	0	0			0
F_6-5282	0	0	0	0	0	0	0
F_6-5283	0	1	0	0	0	1	2
F_6-5284	0	0	0	0	0	0	0
F_6-5285	0	1	0	0	0	1	2
F_6-5286	0	0	0	0			0
F_6-5287	0	1	1	0	0	0	2
F_6-5288	0	0	0	0			0
F_6-5289	0	0	0	0			0
F_6-5290	0	0	0	0			0
F_6-5291	0	0	0	0	0	1	1
F_6-5292	0	1	0	0			1
F_6-5293	0	1	0	0	0	0	1
F_6-5294	0	1	0	0	0	0	1
F_6-5295	0	1	0	0	0	1	2
F_6-5296	0	0	1	0			1
F_6-5297	0	0	0	0	0	1	1
F_6-5298	0	1	1	0	0	0	2
F_6-5299	1	1	1	0			3
F_6-5301	0	0	0	0	0	0	0
F_6-5302	1	1	0	1	0	1	3
F_6-5303	1	1	0	1	0	0	3
F_6-5304	1	0	0	1	0	0	2

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5305	1	0	0	0			1
F_6-5306	0	0	0	0			0
F_6-5307	0	1	0	0			1
F_6-5308	0	0	0	0			0
F_6-5309	0	0	0	0			0
F_6-5310	0	0	0	1			1
F_6-5311	0	1	1	0	0	1	3
F_6-5312	0	1	1	0	0	1	3
F_6-5313	0	0	0	0	0	1	1
F_6-5314	0	1	0	0	0	1	2
F_6-5315	0	0	0	0			0
F_6-5316	0	0	0	0			0
F_6-5317	0	0	0	0			0
F_6-5319	0	0	0	0			0
F_6-5321	0	0	0	0			0
F_6-5322	0	0	0	0			0
F_6-5323	0	0	0	0			0
F_6-5324	0	1	0	0			1
F_6-5325	0	1	0	0			1
F_6-5326	0	0	0	0			0
F_6-5327	0	1	0	0			1
F_6-5328	0	1	0	0			1
F_6-5329	0	0	0	0			0
F_6-5330	0	0	0	0			0
F_6-5331	0	1	0	0			1
F_6-5332	0	0	0	0			0
F_6-5333	0	0	0	0			0

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5334	1	1	0	0			2
F_6-5335	0	0	0	0			0
F_6-5336	0	0	0	1			1
F_6-5337	0	0	0	0			0
F_6-5338	0	0	0	1			1
F_6-5339	0	0	0	0			0
F_6-5340	0	0	0	0			0
F_6-5341	0	1	0	0			1
F_6-5342	0	1	0	0			1
F_6-5343	0	1	1	0	0	0	2
F_6-5344	0	1	0	0	0	0	1
F_6-5345	0	0	0	0	0	0	0
F_6-5346	0	0	0	0			0
F_6-5347	0	0	0	0			0
F_6-5349	0	0	0	0			0
F_6-5350	0	0	1	0			1
F_6-5351	0	0	0	1			1
F_6-5352	0	1	1	0			2
F_6-5353	0	1	1	0			2
F_6-5354	0	1	1	0			2
F_6-5355	0	1	1	0			2
F_6-5356	0	1	1	0			2
F_6-5357	0	1	1	0			2
F_6-5358	0	1	1	0			2
F_6-5359	1	1	1	0			3
F_6-5360	0	1	1	0			2
F_6-5361	0	1	1	0			2

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5362	0	0	1	0	0	0	1
F_6-5363	0	0	0	0			0
F_6-5364	0	0	0	0			0
F_6-5365	0	0	0	0			0
F_6-5366	0	1	0	0			1
F_6-5367	0	0	0	0			0
F_6-5368	0	0	0	0			0
F_6-5369	0	0	0	0			0
F_6-5370	0	0	0	0			0
F_6-5371	0	0	0	0			0
F_6-5372	0	0	1	1	0	0	2
F_6-5373	0	0	0	0			0
F_6-5374	0	0	0	0			0
F_6-5375	0	1	1	0	0	0	2
F_6-5376	0	1	1	1			3
F_6-5379	0	0	0	1			1
F_6-5380	0	1	1	0			2
F_6-5381	0	0	0	1			1
F_6-5384	0	0	0	0	0	0	0
F_6-5385	0	0	0	0	0	0	0
F_6-5386	0	0	0	0	0	0	0
F_6-5387	0	0	0	0	0	0	0
F_6-5388	0	0	0	0			0
F_6-5389	0	0	1	0	0	0	1
F_6-5391	0	1	0	0	0	0	1
F_6-5392	0	0	1	0	0	0	1
F_6-5393	0	0	1	0	0	0	1

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5394	0	0	1	0	0	0	1
F_6-5395	0	1	1	0	0	0	2
F_6-5396	0	0	1	0	0	0	1
F_6-5397	0	1	1	0	0	0	2
F_6-5398	0	0	1	0	0	0	1
F_6-5399	0	1	1	0	0	0	2
F_6-5400	0	1	1	0	0	0	2
F_6-5401	0	1	0	0	0	0	1
F_6-5402	0	1	1	0	0	0	2
F_6-5403	0	1	0	1			2
F_6-5404	1	1	0	1	0	0	2
F_6-5405	0	0	0	1	0	0	1
F_6-5406	1	1	0	1			3
F_6-5407	0	1	0	1	0	0	2
F_6-5408	0	1	0	1	0	0	2
F_6-5409	0	1	0	1	0	0	2
F_6-5410	1	0	0	1	0	1	3
F_6-5411	1	1	0	1	0	0	3
F_6-5412	0	0	0	1	0	1	2
F_6-5413	0	0	0	1	0	1	2
F_6-5414	0	1	0	1	0	0	2
F_6-5415	1	0	0	1	0	0	2
F_6-5416	0	0	0	1	0	0	1
F_6-5417	0	1	0	0			1
F_6-5418	0	1	0	1			2
F_6-5419	0	0	0	0			0
F_6-5420	0	0	0	1			1

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5421	0	0	0	0			0
F_6-5422	0	0	0	0			0
F_6-5423	0	0	0	0	0	0	0
F_6-5424	0	0	0	0	0	0	0
F_6-5425	0	0	0	0	0	1	1
F_6-5426	0	0	0	0	0	0	0
F_6-5427	0	0	0	0	0	1	1
F_6-5428	0	0	0	0	0	1	1
F_6-5429	0	0	0	0	0	0	0
F_6-5430	0	0	0	0	0	1	1
F_6-5431	0	0	0	0	0	1	1
F_6-5432	0	0	0	0			0
F_6-5433	0	0	0	0	0	0	0
F_6-5434	0	0	0	0	0	0	0
F_6-5435	0	0	0	0	0	1	1
F_6-5436	0	0	0	0	0	0	0
F_6-5437	0	0	0	0			0
F_6-5438	0	0	0	0			0
F_6-5439	0	0	0	0	0	0	0
F_6-5440	0	0	0	0	0	0	0
F_6-5441	0	0	0	0			0
F_6-5442	0	0	0	0			0
F_6-5443	0	1	0	0			1
F_6-5444	0	0	0	0	0	1	1
F_6-5445	0	0	0	0	0	1	1
F_6-5446	0	0	0	0			0
F_6-5447	0	0	0	0	0	1	1

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5448	0	0	0	0			0
F_6-5449	0	0	0	0	0	0	0
F_6-5450	0	0	0	0	0	0	0
F_6-5451	0	1	0	0	0	1	2
F_6-5452	0	0	0	0	0	1	1
F_6-5453	0	0	0	0	0	0	0
F_6-5454	0	1	0	0			1
F_6-5455	0	1	0	0			1
F_6-5456	0	0	0	0			0
F_6-5457	0	1	0	0			1
F_6-5458	0	1	0	0			1
F_6-5459	0	1	0	0			1
F_6-5460	0	0	0	0			0
F_6-5461	0	0	0	0			0
F_6-5462	0	1	0	0			1
F_6-5463	0	0	0	0			0
F_6-5465	0	0	0	0			0
F_6-5466	0	0	0	0			0
F_6-5467	0	1	0	0	0	0	1
F_6-5468	0	0	0	0			0
F_6-5469	0	0	0	0			0
F_6-5470	0	0	0	0	0	1	1
F_6-5471	0	0	0	0	0	1	1
F_6-5472	0	0	0	0			0
F_6-5473	0	0	0	0			0
F_6-5474	0	0	0	0			0
F_6-5475	0	0	0	0			0

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5476	0	0	0	0			0
F_6-5477	0	0	1	1			2
F_6-5478	1	0	1	1			3
F_6-5479	0	1	1	1			3
F_6-5480	1	1	1	1	0	1	4
F_6-5481	0	0	0	0			0
F_6-5482	0	1	1	0			2
F_6-5483	1	0	0	0	0	0	1
F_6-5484	0	0	0	0			0
F_6-5485	0	0	0	0			0
F_6-5486	0	1	0	0	0	0	1
F_6-5487	0	0	0	0			0
F_6-5488	0	0	0	1	0	0	1
F_6-5489	0	0	0	0	0	0	0
F_6-5490	0	0	0	0			0
F_6-5491	0	0	0	0			0
F_6-5492	0	0	0	0	0	0	0
F_6-5493	0	0	0	0			0
F_6-5494	0	0	0	0			0
F_6-5495	0	0	0	0			0
F_6-5496	0	0	0	0			0
F_6-5497	0	1	0	0			1
F_6-5498	0	0	0	0			0
F_6-5499	0	0	0	0			0
F_6-5500	0	1	0	0	0	1	2
F_6-5501	0	1	0	0	0	1	2
F_6-5502	0	0	0	0	0	1	1

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5503	0	1	0	0			1
F_6-5504	0	0	0	0			0
F_6-5505	0	1	0	0	0	1	2
F_6-5506	0	0	0	0	0	1	1
F_6-5507	0	0	0	0	0	1	1
F_6-5508	0	1	0	0	0	1	2
F_6-5510	0	1	0	0	0	1	2
F_6-5511	0	1	0	0	0	0	1
F_6-5512	1	0	0	0	0	0	1
F_6-5513	1	0	0	1	0	0	2
F_6-5514	1	0	0	0	0	0	1
F_6-5515	0	0	0	0			0
F_6-5516	1	1	0	0	0	0	2
F_6-5517	1	0	0	0	0	0	1
F_6-5518	0	0	0	0	0	0	0
F_6-5519	0	0	0	0	0	0	0
F_6-5520	0	0	0	0			0
F_6-5521	0	0	0	0			0
F_6-5522	0	0	0	0	0	1	1
F_6-5523	0	0	0	0	0	0	0
F_6-5524	0	0	0	0	0	0	0
F_6-5525	0	0	0	0			0
F_6-5526	0	0	0	0			0
F_6-5527	0	1	0	1			2
F_6-5528	0	0	0	0	0	0	0
F_6-5529	0	0	0	0	0	0	0
F_6-5531	1	1	0	0			2

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5532	1	0	0	0			1
F_6-5533	1	0	0	0			1
F_6-5534	0	0	0	0			0
F_6-5535	0	0	0	0			0
F_6-5537	0	0	0	0			0
F_6-5538	1	0	0	0			1
F_6-5539	0	0	0	0			0
F_6-5540	0	0	0	0			0
F_6-5541	0	0	0	0			0
F_6-5542	1	0	0	0	0	0	1
F_6-5543	1	0	0	0	0	1	2
F_6-5544	1	0	0	1			2
F_6-5545	1	0	0	0			1
F_6-5546	0	0	0	0			0
F_6-5547	0	0	0	0			0
F_6-5548	1	0	0	0			1
F_6-5549	0	0	0	0			0
F_6-5550	0	0	0	0			0
F_6-5551	1	0	0	1			2
F_6-5552	1	0	0	1	0	1	3
F_6-5553	0	0	0	1			1
F_6-5554	0	0	1	0			1
F_6-5555	0	1	0	0			1
F_6-5556	0	0	0	0			0
F_6-5557	0	0	0	0			0
F_6-5558	0	0	0	0			0
F_6-5559	0	0	0	0			0

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5560	0	1	0	0			1
F_6-5561	0	0	0	0			0
F_6-5562	0	0	0	0			0
F_6-5563	1	0	0	0			1
F_6-5564	1	0	0	0			1
F_6-5565	0	0	0	0			0
F_6-5566	0	0	0	0			0
F_6-5567	0	0	0	0			0
F_6-5568	0	0	0	0			0
F_6-5569	0	1	0	0			1
F_6-5570	0	0	0	0			0
F_6-5571	0	0	0	0			0
F_6-5572	0	0	0	0			0
F_6-5573	0	1	1	0			2
F_6-5574	0	0	0	0			0
F_6-5575	0	0	0	0			0
F_6-5576	0	0	0	0			0
F_6-5577	0	0	0	0			0
F_6-5578	0	0	0	0			0
F_6-5579	0	0	0	0			0
F_6-5581	0	0	0	0			0
F_6-5582	0	0	0	0			0
F_6-5583	0	0	0	0			0
F_6-5584	0	0	0	0			0
F_6-5585	0	0	0	0			0
F_6-5586	0	1	0	0			1
F_6-5587	1	0	0	1			2

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5588	1	1	1	0	0	1	4
F_6-5589	0	0	0	0			0
F_6-5590	0	0	0	0			0
F_6-5591	0	0	0	0			0
F_6-5592	0	0	0	0			0
F_6-5593	0	0	0	0			0
F_6-5594	0	1	0	1			2
F_6-5595	0	0	0	0			0
F_6-5596	0	0	0	0	0	0	0
F_6-5597	0	0	0	0	0	0	0
F_6-5598	0	0	1	0			1
F_6-5599	0	0	0	0			0
F_6-5601	0	1	1	0	0	1	3
F_6-5602	0	0	0	1	0	1	2
F_6-5603	0	0	0	1	0	0	1
F_6-5604	0	0	0	0	0	1	1
F_6-5605	0	0	0	0	0	1	1
F_6-5606	0	1	0	1			2
F_6-5607	0	0	0	1			1
F_6-5608	0	0	0	1			1
F_6-5609	0	0	0	1	0	0	1
F_6-5610	0	0	0	1			1
F_6-5612	0	0	0	0			0
F_6-5613	0	0	0	0			0
F_6-5614	0	1	0	0			1
F_6-5615	0	0	0	0			0
F_6-5617	0	0	0	0			0

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5618	0	0	0	0			0
F_6-5619	0	0	0	0	0	1	1
F_6-5620	0	0	0	0	0	1	1
F_6-5621	0	0	0	0	0	1	1
F_6-5622	0	0	0	0			0
F_6-5623	0	0	0	0	0	1	1
F_6-5624	0	0	0	0			0
F_6-5625	0	0	0	0	0	0	0
F_6-5626	0	0	0	0			0
F_6-5627	0	0	0	0			0
F_6-5628	0	0	0	0	0	0	0
F_6-5629	0	0	0	0			0
F_6-5630	0	0	0	0			0
F_6-5632	0	0	0	0	0	1	1
F_6-5633	0	0	0	0			0
F_6-5634	0	0	0	0			0
F_6-5635	0	1	0	0			1
F_6-5636	0	0	0	0	0	0	0
F_6-5637	0	0	0	0	0	0	0
F_6-5638	0	0	0	0	0	0	0
F_6-5639	0	0	0	0	0	0	0
F_6-5640	0	0	0	0	0	0	0
F_6-5641	0	0	0	0	0	0	0
F_6-5642	0	0	0	0			0
F_6-5643	0	0	0	0			0
F_6-5644	0	0	0	0			0
F_6-5645	0	0	0	0	0	1	1

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5646	0	0	0	0			0
F_6-5647	0	0	0	0			0
F_6-5648	0	0	0	0			0
F_6-5649	0	0	0	0	0	0	0
F_6-5650	0	0	0	0	0	1	1
F_6-5651	0	0	0	0			0
F_6-5652	0	0	0	0			0
F_6-5653	0	0	0	0			0
F_6-5654	0	0	0	0	0	1	1
F_6-5655	0	0	0	0	0	1	1
F_6-5656	0	0	0	0			0
F_6-5657	0	1	0	0	0	1	2
F_6-5658	0	0	0	0	0	1	1
F_6-5659	0	0	0	0			0
F_6-5660	0	1	0	0	0	1	2
F_6-5661	0	1	0	0	0	1	2
F_6-5662	0	0	0	0			0
F_6-5663	0	1	0	0	0	1	2
F_6-5664	1	0	0	0			1
F_6-5665	1	0	0	0			1
F_6-5667	0	0	0	1			1
F_6-5668	1	0	0	0			1
F_6-5669	1	0	0	0			1
F_6-5670	1	0	0	0			1
F_6-5671	0	0	0	0			0
F_6-5672	0	0	0	0			0
F_6-5673	1	0	0	0			1

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5674	0	0	0	1			1
F_6-5675	0	0	0	1			1
F_6-5676	1	1	0	1			3
F_6-5677	0	1	0	1	0	0	2
F_6-5678	0	1	0	1	0	1	3
F_6-5679	0	1	1	0	0	0	2
F_6-5680	0	1	0	0			1
F_6-5681	0	0	0	0			0
F_6-5682	0	0	0	0			0
F_6-5683	0	0	0	0			0
F_6-5684	0	1	0	1	0	1	3
F_6-5685	0	1	0	1			2
F_6-5686	0	1	0	1			2
F_6-5687	0	1	0	0			1
F_6-5688	1	1	0	1	0	1	4
F_6-5689	1	0	0	0	0	0	1
F_6-5690	0	0	0	0	0	0	0
F_6-5691	0	0	0	0	0	0	0
F_6-5692	1	0	0	0			1
F_6-5693	0	0	0	0	0	0	0
F_6-5694	0	0	0	0			0
F_6-5695	0	1	0	0			1
F_6-5696	0	0	0	0			0
F_6-5697	0	0	0	0	0	1	1
F_6-5698	0	1	0	0	0	1	2
F_6-5699	0	0	0	0	0	0	0
F_6-5700	0	0	0	0			0

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5701	0	1	0	0			1
F_6-5702	0	1	0	0	0	0	1
F_6-5703	0	1	0	0	0	1	2
F_6-5704	0	0	0	0	0	1	1
F_6-5705	0	0	0	0	0	1	1
F_6-5706	0	0	0	1			1
F_6-5707	0	0	0	0			0
F_6-5708	0	0	0	0			0
F_6-5709	0	0	0	0	0	1	1
F_6-5710	0	0	0	0	0	1	1
F_6-5711	0	0	0	0	0	1	1
F_6-5712	0	0	0	0			0
F_6-5713	0	0	0	0			0
F_6-5714	0	0	0	0			0
F_6-5715	1	0	0	1	0	1	3
F_6-5716	0	0	0	0			0
F_6-5717	0	0	0	0	0	0	0
F_6-5718	0	0	0	0			0
F_6-5720	1	0	0	0			1
F_6-5721	1	0	0	0	0	0	1
F_6-5722	0	0	0	0			0
F_6-5723	0	0	0	0			0
F_6-5724	0	0	0	0			0
F_6-5725	0	0	0	0			0
F_6-5726	0	0	0	0	0	1	1
F_6-5727	0	0	0	0			0
F_6-5728	1	0	0	0			1

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5729	1	0	0	0	0	0	1
F_6-5730	0	0	0	0			0
F_6-5731	0	0	0	0			0
F_6-5732	0	0	0	0			0
F_6-5733	0	0	0	0			0
F_6-5734	0	0	0	0			0
F_6-5735	0	0	0	0			0
F_6-5736	0	0	0	0			0
F_6-5737	0	0	0	0			0
F_6-5738	0	0	0	0	0	1	1
F_6-5739	0	0	0	0	0	1	1
F_6-5741	0	0	0	1	0	0	1
F_6-5742	0	0	0	1			1
F_6-5743	0	0	0	0			0
F_6-5744	0	0	0	0			0
F_6-5745	0	1	1	0			2
F_6-5746	0	0	0	1			1
F_6-5748	0	0	0	0			0
F_6-5749	0	0	0	0			0
F_6-5750	0	0	0	0			0
F_6-5751	1	0	0	1	0	1	3
F_6-5752	0	0	0	0			0
F_6-5753	1	0	0	1			2
F_6-5754	1	0	0	1			2
F_6-5755	0	0	0	0			0
F_6-5756	0	0	0	0			0
F_6-5757	1	0	0	0	0	0	1

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5758	0	0	0	0	0	0	0
F_6-5759	0	0	0	0			0
F_6-5760	0	0	0	0			0
F_6-5761	0	0	0	0			0
F_6-5762	0	0	0	0	0	1	1
F_6-5763	0	0	0	0			0
F_6-5764	0	0	0	0			0
F_6-5765	0	0	0	0			0
F_6-5766	0	0	0	0			0
F_6-5767	1	0	0	1			2
F_6-5768	0	0	0	1			1
F_6-5769	0	0	0	1			1
F_6-5770	0	0	0	1			1
F_6-5771	0	0	0	1			1
F_6-5772	1	0	0	1			2
F_6-5773	0	0	0	0			0
F_6-5774	0	0	0	0			0
F_6-5775	0	0	0	0			0
F_6-5776	0	0	1	0	0	1	2
F_6-5777	0	0	0	0			0
F_6-5778	0	0	0	0			0
F_6-5780	0	0	0	0			0
F_6-5781	0	0	0	0			0
F_6-5782	1	0	0	1			2
F_6-5783	0	0	0	0			0
F_6-5786	0	0	0	0			0
F_6-5789	0	0	0	0			0

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	*Sr24*	*Lr34*	*Lr37*	*Sr26*	*Sr31*	
F_6-5790	0	0	0	0			0
F_6-5791	0	0	0	0			0
F_6-5793	0	0	0	0			0
F_6-5794	0	0	0	0			0
FHB-6402	0	1	0	0	0	0	1
FHB-6403	0	0	0	1	0	0	1
FHB-6404	0	1	0	0	0	0	1
FHB-6405	0	0	0	1	0	0	1
FHB-6407	0	1	0	0	0	0	1
FHB-6408	0	1	0	1	0	0	2
FHB-6409	0	1	0	1	0	0	2
FHB-6410	0	0	0	1	0	0	1
FHB-6411	0	0	0	1	0	0	1
FHB-6412	0	0	0	1	0	0	1
FHB-6413	0	0	0	1	0	0	1
FHB-6414	0	0	0	1	0	0	1
FHB-6415	0	0	0	1	0	0	1
FHB-6416	0	0	0	1	0	0	1
FHB-6417	0	1	0	1	0	0	2
FHB-6418	0	1	1	1	0	0	3
FHB-6419	0	1	0	0	0	0	1
FHB-6420	0	0	0	1	0	0	1
FHB-6422	0	0	0	1	0	0	1
FHB-6423	0	0	0	1	0	0	1
FHB-6424	0	0	0	0	0	0	0
FHB-6426	0	0	0	1	0	0	1
FHB-6427	0	0	0	1	0	0	2

Donor lines	Rust resistance genes						Total rust resistance genes per line
	Sr2	***Sr24***	***Lr34***	***Lr37***	***Sr26***	***Sr31***	
FHB-6428	0	0	0	1	0	0	2
FHB-6429	0	0	0	0	0	0	0
FHB-6430	0	0	0	1	0	0	1
FHB-6431	0	0	1	0	0	0	1
FHB-6432	0	0	0	0	0	0	0
FHB-6433	0	0	0	0	0	0	0
FHB-6434	0	0	0	0	0	0	0
FHB-6435	0	0	0	0	0	0	0

0: The gene is not present

1: The gene is present

The line was not screened for a specific gene

Table A.2.The baking quality and yield marker molecular data of the wheat lines and the controls that were included in the FHB phenotyping trials.

Wheat line	*GluDx*			*Ppd-D1-2D*		*TaGS-D1-D7*	*TaGW2-6B*	*TaGS5-3A*	Total amount of markers per line
	Dy10	*Dy12*	*Dx5*	228 bp	414 bp				
F_6-5181	1	1	1	1	0	0	0	1	5
F_6-5183	1	1	1	1	0	0	0	1	5
F_6-5191	0	1	0	1	0	1	0	1	4
F_6-5192	0	1	0	1	0	1	0	1	4
F_6-5195	0	1	0	1	0	0	0	1	3
F_6-5198	0	1	0	1	0	0	0	1	3
F_6-5200	0	1	0	1	0	0	0	1	3
F_6-5225	0	1	0	1	0	1	0	1	4
F_6-5243	1	1	1	1	0	1	0	1	6
F_6-5244	0	1	0	1	0	0	0	1	3
F_6-5297	1	1	1	1	0	0	0	1	4
F_6-5301	0	1	0	1	0	1	1	1	5
F_6-5397	0	1	0	1	0	1	1	0	4
F_6-5399	0	1	0	1	0	1	1	0	4

Wheat line	*GluDx*			*Ppd-D1-2D*		*TaGS-D1-D7*	*TaGW2-6B*	*TaGS5-3A*	Total amount of markers per line
	Dy10	*Dy12*	*Dx5*	228 bp	414 bp				
F_6-5405	0	1	0	1	0	1	0	1	4
F_6-5411	0	1	0	1	0	1	0	1	4
F_6-5426	0	1	0	1	0	1	0	1	4
F_6-5429	1	1	0	1	0	1	1	1	6
F_6-5433	0	1	0	1	0	0	1	1	4
F_6-5439	0	1	0	1	0	0	0	1	3
F_6-5440	0	1	0	1	0	0	0	1	3
F_6-5450	0	1	1	1	0	1	1	1	6
F_6-5451	0	0	1	1	0	0	1	1	4
F_6-5453	0	1	0	1	0	0	0	1	3
F_6-5523	0	1	0	1	0	1	1	1	5
F_6-5596	0	1	0	1	0	1	1	1	5
F_6-5636	1	1	1	1	0	0	1	1	6
F_6-5657	1	1	1	1	0	1	1	1	7
F_6-5661	1	1	1	1	0	1	1	1	7
F_6-5704	1	1	1	1	0	0	1	1	6

Wheat line	*GluDx*			*Ppd-D1-2D*		*TaGS-D1-D7*	*TaGW2-6B*	*TaGS5-3A*	Total amount of markers per line
	Dy10	*Dy12*	*Dx5*	228 bp	414 bp				
FHB-6402	0	0	1	0	1	1	0	0	3
FHB-6403	1	1	1	1	0	1	0	0	5
FHB-6404	1	1	1	0	1	1	1	0	3
FHB-6405	1	1	0	1	0	0	0	0	6
FHB-6407	1	1	1	1	0	1	0	0	5
FHB-6408	1	1	1	1	0	1	1	0	6
FHB-6409	0	0	1	1	0	0	0	0	2
FHB-6410	1	1	0	1	0	1	0	0	4
FHB-6411	1	1	1	1	0	1	1	0	6
FHB-6412	0	1	1	1	0	1	0	0	4
FHB-6413	0	1	1	1	0	1	0	0	4
FHB-6414	0	1	1	1	0	1	0	0	4
FHB-6415	1	1	1	1	0	1	0	0	5
FHB-6416	1	1	1	0	1	1	0	0	5
FHB-6417	1	1	1	1	0	1	0	0	5
FHB-6418	1	1	0	1	0	0	1	1	5

Wheat line	*GluDx*			*Ppd-D1-2D*		*TaGS-D1-D7*	*TaGW2-6B*	*TaGS5-3A*	Total amount of markers per line
	Dy10	*Dy12*	*Dx5*	228 bp	414 bp				
FHB-6419	0	0	0	1	0	0	0	0	1
FHB-6420	0	0	1	1	0	1	0	0	3
FHB-6422	1	1	0	1	0	0	1	1	5
FHB-6423	0	1	1	1	0	1	0	0	4
FHB-6424	0	0	0	0	1	0	1	1	3
FHB-6426	0	1	1	1	0	1	0	0	4
FHB-6427	0	1	1	1	0	1	1	1	6
FHB-6428	1	1	0	1	0	0	1	1	5
FHB-6429	1	0	0	1	0	1	0	0	3
FHB-6430	0	0	0	0	1	0	1	1	3
FHB-6431	1	1	1	1	0	1	0	0	5
FHB-6432	1	1	1	1	0	1	0	0	5
FHB-6433	1	1	1	1	0	1	0	0	5
FHB-6434	0	1	1	1	0	1	0	0	4
FHB-6435	1	1	0	1	0	1	0	0	4
Sumai3	1	1	1	1	1	1	1	0	3

Wheat line	*GluDx*			*Ppd-D1-2D*		*TaGS-D1-D7*	*TaGW2-6B*	*TaGS5-3A*	Total amount of markers per line
	Dy10	***Dy12***	***Dx5***	**228 bp**	**414 bp**				
PAN4371	1	0	0	1	0	1	0	1	4

Table A.3. The FHB resistance molecular data of the wheat lines and controls that were included in the FHB phenotyping trials.

Selected Donor Lines	FHB Resistance QTL's							Total FHB resistance QTL's per line
	Qfhs.ifa-5A		*7AQTL*		*Qfhs.ndsu-3BS*			
	gwm304	*gwm293*	*gwm130*	*gwm233*	*gwm493*	*gwm533*	*Barc133*	
F_6-5181	0	0	0	0	0	0	0	0
F_6-5183	0	0	0	0	0	0	0	0
F_6-5191	0	0	0	0	0	0	0	0
F_6-5192	0	0	0	0	0	0	0	0
F_6-5195	0	0	0	0	0	0	0	0
F_6-5198	0	0	0	0	0	0	0	0
F_6-5200	0	0	0	0	0	0	0	0
F_6-5225	0	0	1	0	0	0	0	0
F_6-5243	0	0	1	0	0	0	0	0
F_6-5244	0	0	0	0	0	0	0	0
F_6-5297	0	0	1	0	0	0	0	0
F_6-5301	0	0	0	0	0	0	0	0
F_6-5397	0	0	0	0	1	0	0	0
F_6-5399	0	0	0	0	0	0	0	0

Selected Donor Lines	FHB Resistance QTL's							Total FHB resistance QTL's per line
	Qfhs.ifa-5A		*7AQTL*		*Qfhs.ndsu-3BS*			
	gwm304	*gwm293*	*gwm130*	*gwm233*	*gwm493*	*gwm533*	*Barc133*	
F_6-5405	0	0	0	0	0	0	0	0
F_6-5411	0	0	0	0	0	0	0	0
F_6-5426	0	0	0	0	0	0	0	0
F_6-5429	0	0	0	0	1	0	0	0
F_6-5433	0	0	0	0	1	0	0	0
F_6-5439	0	0	0	0	0	0	0	0
F_6-5440	0	0	0	0	0	0	0	0
F_6-5450	0	0	0	0	1	0	0	0
F_6-5451	0	0	1	0	0	0	0	0
F_6-5453	0	0	0	0	1	1	0	1
F_6-5523	0	0	0	0	0	0	0	0
F_6-5596	0	0	0	0	0	0	0	0
F_6-5636	0	0	1	0	0	0	1	0
F_6-5657	0	0	0	0	1	0	0	0
F_6-5661	0	0	0	0	0	0	0	0
F_6-5704	0	0	0	0	1	0	0	0

Selected Donor Lines	FHB Resistance QTL's							Total FHB resistance QTL's per line
	Qfhs.ifa-5A		*7AQTL*		*Qfhs.ndsu-3BS*			
	gwm304	*gwm293*	*gwm130*	*gwm233*	*gwm493*	*gwm533*	*Barc133*	
FHB-6402	0	0	0	0	0	0	0	0
FHB-6403	0	0	0	0	0	0	0	0
FHB-6404	0	0	0	0	0	0	0	0
FHB-6405	0	0	0	0	0	1	1	0
FHB-6407	1	0	0	1	0	0	0	0
FHB-6408	0	0	0	0	0	0	0	0
FHB-6409	0	0	0	0	0	0	0	0
FHB-6410	0	0	1	0	0	0	1	0
FHB-6411	0	0	0	0	0	0	0	0
FHB-6412	0	0	0	0	0	0	0	0
FHB-6413	0	0	0	0	0	0	0	0
FHB-6414	0	0	0	0	0	0	0	0
FHB-6415	0	0	0	0	0	0	0	0
FHB-6416	0	0	0	0	0	0	0	0
FHB-6417	0	0	0	0	0	0	0	0
FHB-6418	0	0	0	0	0	0	0	0

Selected Donor Lines	FHB Resistance QTL's							Total FHB resistance QTL's per line
	Qfhs.ifa-5A		*7AQTL*		*Qfhs.ndsu-3BS*			
	gwm304	*gwm293*	*gwm130*	*gwm233*	*gwm493*	*gwm533*	*Barc133*	
FHB-6419	0	0	1	0	0	0	0	0
FHB-6420	0	0	0	0	0	0	0	0
FHB-6422	0	0	0	0	0	0	0	0
FHB-6423	0	0	1	1	0	0	0	1
FHB-6424	0	0	0	0	0	0	0	0
FHB-6426	0	0	1	0	0	0	0	0
FHB-6427	0	0	0	0	1	0	0	0
FHB-6428	0	0	0	0	1	0	1	0
FHB-6429	1	0	0	0	0	0	0	0
FHB-6430	0	0	0	0	0	0	0	0
FHB-6431	0	0	0	0	0	0	0	0
FHB-6432	0	0	0	0	0	0	0	0
FHB-6433	0	0	0	0	0	0	0	0
FHB-6434	0	0	0	0	0	0	0	0
FHB-6435	0	1	0	0	0	0	0	0
Sumai3	1	1	1	1	1	1	1	3

Selected Donor Lines	FHB Resistance QTL's							Total FHB resistance QTL's per line
	Qfhs.ifa-5A		*7AQTL*		*Qfhs.ndsu-3BS*			
	gwm304	*gwm293*	*gwm130*	*gwm233*	*gwm493*	*gwm533*	*Barc133*	
PAN4371	0	0	0	0	0	0	0	0

Table A.4. The screening of the female population of MS-MARS cycle 1 for rust resistance genes.

Female Lines	Rust resistance genes		Total number of genes per line
	Sr2	*Lr34*	
F_0-1	0	1	1
F_0-2	0	0	0
F_0-3	0	0	0
F_0-4	0	0	0
F_0-5	0	0	0
F_0-6	0	0	0
F_0-7	0	0	0
F_0-8	1	0	1
F_0-9	0	0	0
F_0-10	1	1	2
F_0-11	0	0	0
F_0-12	1	0	1
F_0-13	0	0	0
F_0-14	0	0	0
F_0-15	0	0	0
F_0-16	0	0	0
F_0-17	0	0	0
F_0-18	1	0	1
F_0-19	0	0	0
F_0-20	1	0	1
F_0-21	1	0	1
F_0-22	0	0	0
F_0-23	0	1	1
F_0-24	1	0	1
F_0-25	0	0	0
F_0-26	1	0	1
F_0-27	0	1	1
F_0-28	0	0	0
F_0-29	1	1	2
F_0-30	1	0	1
F_0-31	1	1	2
F_0-32	1	1	2
F_0-33	0	0	0
F_0-34	0	0	0
F_0-35	0	1	1

Table A.5. The screening of the female population of MS-MARS cycle 2 for rust resistance genes.

Female Lines	Rust resistance genes		Total number of genes per line
	Sr2	*Lr34*	
F_1-1	0	0	0
F_1-2	0	1	1
F_1-3	0	0	0
F_1-4	1	1	2
F_1-5	1	0	1
F_1-6	0	1	1
F_1-7	0	0	0
F_1-8	0	0	0
F_1-9	0	1	1
F_1-10	0	0	0
F_1-11	0	0	0
F_1-12	0	0	0
F_1-13	0	0	0
F_1-14	1	1	2
F_1-15	0	0	0
F_1-16	0	0	0
F_1-17	0	0	0
F_1-18	1	0	1
F_1-19	1	0	1
F_1-20	0	1	1
F_1-21	0	1	1
F_1-22	0	0	0
F_1-23	0	1	1
F_1-24	0	1	1
F_1-25	0	0	0
F_1-26	1	1	2
F_1-27	1	1	2
F_1-28	1	1	2
F_1-29	1	0	1
F_1-30	0	0	0
F_1-31	0	0	0
F_1-32	1	0	1
F_1-33	1	0	1
F_1-34	1	1	2
F_1-35	0	1	1

Female Lines	Rust resistance genes		Total number of genes per line
	Sr2	*Lr34*	
F_0-36	0	1	1
F_0-37	0	1	1
F_0-38	0	0	0
F_0-39	1	1	2
F_0-40	0	0	0
F_0-41	0	0	0
F_0-42	0	0	0
F_0-43	1	1	2
F_0-44	0	0	0
F_0-45	0	0	0
F_0-46	0	1	1
F_0-47	0	0	0
F_0-48	0	1	1
F_0-49	0	0	0
F_0-50	0	0	0
F_0-51	0	0	0
F_0-52	1	0	1
F_0-53	0	0	0
F_0-54	0	1	1
F_0-55	0	0	0
F_0-56	0	0	0
F_0-57	0	0	0
F_0-58	0	0	0
F_0-59	0	0	0
F_0-60	0	0	0
F_0-61	0	0	0
F_0-62	1	0	1
F_0-63	0	0	0
F_0-64	0	1	1
F_0-65	0	0	0
F_0-66	0	0	0
F_0-67	0	0	0
F_0-68	0	0	0
F_0-69	0	0	0
F_0-70	0	0	0
F_0-71	1	1	2
F_0-72	0	1	1
F_0-73	0	1	1
F_0-74	0	0	0
F_0-75	1	0	1

Female Lines	Rust resistance genes		Total number of genes per line
	Sr2	*Lr34*	
F_1-36	0	0	0
F_1-37	1	1	2
F_1-38	1	1	2
F_1-39	1	1	2
F_1-40	1	0	1
F_1-41	1	0	1
F_1-42	0	0	0
F_1-43	0	0	0
F_1-44	1	1	2
F_1-45	0	0	0
F_1-46	0	0	0
F_1-47	0	0	0
F_1-48	1	1	2
F_1-49	0	0	0
F_1-50	0	1	1
F_1-51	0	1	1
F_1-52	0	1	1
F_1-53	1	1	2
F_1-54	0	1	1
F_1-55	0	0	0
F_1-56	1	1	2
F_1-57	0	1	1
F_1-58	0	0	0
F_1-59	0	1	1
F_1-60	0	0	0
F_1-61	1	0	1
F_1-62	1	0	1
F_1-63	0	1	1
F_1-64	1	0	1
F_1-65	1	0	1
F_1-66	0	1	1
F_1-67	0	1	1
F_1-68	0	1	1
F_1-69	0	0	0
F_1-70	0	1	1
F_1-71	1	0	1
F_1-72	0	0	0
F_1-73	0	0	0
F_1-74	0	0	0
F_1-75	0	0	0

Female Lines	Rust resistance genes		Total number of genes per line
	Sr2	*Lr34*	
F_0-76	0	0	0
F_0-77	0	0	0
F_0-78	0	1	1
F_0-79	0	0	0
F_0-80	1	1	2
F_0-81	1	0	1
F_0-82	0	1	1
F_0-83	0	0	0
F_0-84	0	0	0
F_0-85	0	1	1
F_0-86	1	1	2
F_0-87	0	0	0
F_0-88	0	0	0
F_0-89	0	0	0
F_0-90	0	0	0
F_0-91	1	1	2
F_0-92	1	1	2
F_0-93	0	0	0
F_0-94	0	0	0
F_0-95	1	0	1
F_0-96	0	0	0
F_0-97	0	0	0
F_0-98	0	0	0
F_0-99	0	0	0
F_0-100	1	0	1
F_0-101	0	0	0
F_0-102	0	1	1
F_0-103	1	0	1
F_0-104	1	0	1
F_0-105	0	1	1
F_0-106	0	1	1
F_0-107	1	0	1
F_0-108	1	0	1
F_0-109	0	0	0
F_0-110	1	0	1
F_0-111	1	0	1
F_0-112	0	1	1
F_0-113	1	0	1
F_0-114	0	0	0
F_0-115	1	0	1
F_0-116	0	1	1

Female Lines	Rust resistance genes		Total number of genes per line
	Sr2	*Lr34*	
F_1-76	0	1	1
F_1-77	1	0	1
F_1-78	1	0	1
F_1-79	0	0	0
F_1-80	0	0	0
F_1-81	0	0	0
F_1-82	1	0	1
F_1-83	0	0	0
F_1-84	0	0	0
F_1-85	0	0	0
F_1-86	0	0	0
F_1-87	0	1	1
F_1-88	1	1	2
F_1-89	0	1	1
F_1-90	0	0	0
F_1-91	0	0	0
F_1-92	0	0	0
F_1-93	0	0	0
F_1-94	0	0	0
F_1-95	1	0	1
F_1-96	0	0	0
F_1-97	0	0	0
F_1-98	0	0	0
F_1-99	0	1	1
F_1-100	1	0	1
F_1-101	1	0	1
F_1-102	0	0	0
F_1-103	0	1	1
F_1-104	0	0	0
F_1-105	0	0	0
F_1-106	0	1	1
F_1-107	1	1	2
F_1-108	0	0	0
F_1-109	0	0	0
F_1-110	0	0	0
F_1-111	0	0	0
F_1-112	1	1	2
F_1-113	0	0	0
F_1-114	0	0	0
F_1-115	0	1	1
F_1-116	0	0	0

Female Lines	Rust resistance genes		Total number of genes per line
	Sr2	***Lr34***	
F_0-117	1	0	1
F_0-118	0	0	0
F_0-119	0	0	0
F_0-120	0	0	0

Female Lines	Rust resistance genes		Total number of genes per line
	Sr2	***Lr34***	
F_1-117	0	0	0
F_1-118	0	1	1
F_1-119	0	0	0
F_1-120	1	0	1

Addendum B

Table B.1. Screening molecularly and phenotypically for the male sterility gene in the female population of 2020.

Wheat line	*Ms3*	Phenotyping
F_1-1	1	Sterile
F_1-2	1	Sterile
F_1-3	0	Fertile
F_1-4	0	Fertile
F_1-5	0	Sterile
F_1-6	1	Fertile
F_1-7	0	Sterile
F_1-8	1	Fertile
F_1-9	0	Fertile
F_1-10	0	Fertile
F_1-11	0	Fertile
F_1-12	0	Sterile
F_1-13	1	Fertile
F_1-14	0	Fertile
F_1-15	1	Sterile
F_1-16	1	Fertile
F_1-17	1	Sterile
F_1-18	0	Sterile
F_1-19	1	Fertile
F_1-20	0	Fertile
F_1-21	0	Fertile
F_1-22	0	Sterile
F_1-23	1	Sterile
F_1-24	0	Fertile
F_1-25	0	Fertile
F_1-26	0	Sterile
F_1-27	0	Fertile
F_1-28	0	Sterile
F_1-29	0	Sterile
F_1-30	0	Fertile
F_1-31	1	Fertile
F_1-32	0	Fertile
F_1-33	0	Fertile
F_1-34	0	Sterile
F_1-35	1	Sterile
F_1-36	0	Fertile
F_1-37	1	Sterile
F_1-38	0	Sterile

Wheat line	*Ms3*	Phenotyping
F_1-39	0	Fertile
F_1-40	1	Fertile
F_1-41	1	Fertile
F_1-42	0	Fertile
F_1-43	1	Sterile
F_1-44	1	Fertile
F_1-45	0	Sterile
F_1-46	1	Sterile
F_1-47	0	Sterile
F_1-48	0	Sterile
F_1-49	1	Fertile
F_1-50	1	Sterile
F_1-51	1	Fertile
F_1-52	0	Fertile
F_1-53	1	Fertile
F_1-54	1	Fertile
F_1-55	0	Sterile
F_1-56	1	Fertile
F_1-57	0	Sterile
F_1-58	1	Fertile
F_1-59	1	Sterile
F_1-60	1	Sterile
F_1-61	0	Fertile
F_1-62	1	Fertile
F_1-63	1	Sterile
F_1-64	0	Fertile
F_1-65	0	Fertile
F_1-66	0	Fertile
F_1-67	0	Fertile
F_1-68	0	Sterile
F_1-69	1	Sterile
F_1-70	0	Fertile
F_1-71	0	Fertile
F_1-72	0	Fertile
F_1-73	0	Fertile
F_1-74	1	Sterile
F_1-75	0	Fertile
F_1-76	0	Fertile

Wheat line	*Ms3*	Phenotyping
F_1-77	1	Sterile
F_1-78	1	Fertile
F_1-79	1	Sterile
F_1-80	0	Fertile
F_1-81	1	Fertile
F_1-82	0	Sterile
F_1-83	0	Fertile
F_1-84	0	Fertile
F_1-85	0	Fertile
F_1-86	0	Fertile
F_1-87	0	Sterile
F_1-88	1	Fertile
F_1-89	1	Fertile
F_1-90	1	Sterile
F_1-91	1	Fertile
F_1-92	1	Sterile
F_1-93	0	Sterile
F_1-94	0	Sterile
F_1-95	1	Fertile
F_1-96	0	Sterile
F_1-97	1	Fertile
F_1-98	0	Fertile
F_1-99	0	Fertile
F_1-100	0	Sterile
F_1-101	0	Sterile
F_1-102	1	Sterile
F_1-103	0	Fertile
F_1-104	0	Sterile
F_1-105	0	Sterile
F_1-106	1	Fertile
F_1-107	0	Fertile
F_1-108	0	Fertile
F_1-109	0	Sterile
F_1-110	1	Sterile
F_1-111	0	Fertile
F_1-112	1	Sterile
F_1-113	1	Sterile
F_1-114	0	Sterile
F_1-115	1	Fertile
F_1-116	0	Sterile
F_1-117	1	Fertile
F_1-118	0	Sterile
F_1-119	1	Fertile

Wheat line	*Ms3*	Phenotyping
F_1-120	0	Fertile

Addendum C

Table C1. The rust field phenotyping data recorded.

			Rust resistance genes screened for						
Groups	**Wheat line**	**Selection history**	***Sr2***	***Sr24***	***Lr34***	***Lr37***	**Sr26**	**Sr31**	**Pt response**
Group 1	F_6-5150	13H306-4-4-1	0	0	0	0	0	0	TMR
	F_6-5152	13H306-4-4-3	0	0	0	0	0	0	TMR
	F_6-5153	13H306-4-4-4	0	0	0	0	0	0	TMR
Group 2	F_6-5221	13H317-1-1-1-1	1	0	0	1	0	1	20S
	F_6-5222	13H306-5-1-1-2	0	0	0	0	0	1	20S
	F_6-5223	13H317-1-1-1-3	0	0	0	1	0	1	20S
	F_6-5224	13H317-1-1-1-4	0	0	0	0	0	1	20S
	F_6-5225	13H317-1-1-1-5	0	1	1	0	0	0	20S
	F_6-5226	13H317-1-1-1-6	0	1	0	0	0	1	40S
	F_6-5229	13H317-1-1-1-9	0	0	0	0	0	1	20S
	F_6-5230	13H317-1-1-1-10	0	1	0	0	0	1	20S
Group 3	F_6-5291	13H271-2-1-1	1	0	0	1	0	1	20S
	F_6-5293	13H271-2-1-3	0	0	0	0	0	0	20S
	F_6-5295	13H271-2-1-5	0	1	0	0	0	0	20S
Group 4	F_6-5296	11H148-4-4-1-7-1	0	1	0	0	0	1	R
	F_6-5297	11H148-4-4-1-7-2	0	1	1	0	0	1	R
	F_6-5298	11H148-4-4-1-7-3	0	1	0	0	0	0	R
Group 5	F_6-5301	13H273-3-11-1	1	0	0	1	0	0	40S
	F_6-5302	13H273-3-11-2	0	0	0	0	0	1	40S
	F_6-5303	13H273-3-11-3	0	0	0	1	0	0	40S
	F_6-5304	13H273-3-11-4	0	1	1	0	0	0	40S

			Rust resistance genes screened for						
Groups	**Wheat line**	**Selection history**	***Sr2***	***Sr24***	***Lr34***	***Lr37***	**Sr26**	**Sr31**	**LR response**
Group 6	F_6-5413	13H099-9-1	0	1	0	0	0	1	40S
	F_6-5414	13H099-9-2	0	0	1	0	0	0	40S
	F_6-5415	13H099-9-3	0	1	0	0	0	0	40S
	F_6-5416	13H099-9-4	0	0	0	0	0	0	40S
Group 7	F_6-5517	13H308-2-5-1	0	0	0	0	0	0	40S
	F_6-5518	13H308-2-5-2	0	0	0	0	0	0	40S
	F_6-5519	13H308-2-5-3	0	1	0	0	0	0	40S
Group 8	F_6-5522	13H308-2-5-6	0	0	0	0	0	1	40S
	F_6-5523	13H308-2-5-7	0	1	0	0	0	0	40S
	F_6-5524	13H308-2-5-8	0	1	1	0	0	0	40S
Group 9	F_6-5660	13H306-8-1-2	0	1	0	0	0	1	20S
	F_6-5661	13H306-8-1-3	0	0	1	0	0	1	20S
	F_6-5663	13H306-8-1-5	1	0	0	0	0	1	20S
Group 10	F_6-5689	13H273-2-12-1	0	0	1	0	0	0	20S
	F_6-5690	13H273-2-12-2	1	1	0	1	0	0	20S
	F_6-5691	13H273-2-12-3	1	0	0	0	0	0	40S
	F_6-5693	13H273-2-12-5	1	0	0	1	0	0	40S
	F_6-5697	13H275-2-8-4	1	0	0	1	0	1	40S
	F_6-5698	13H275-2-8-5	0	1	0	1	0	1	40S
Group 11	F_6-5709	13H306-9-5-1	0	1	1	0	0	1	20S
	F_6-5710	13H306-9-5-2	0	1	0	0	0	1	20S
	F_6-5711	13H306-9-5-3	0	1	0	0	0	1	40S

			Rust resistance genes screened for						
Groups	**Wheat line**	**Selection history**	***Sr2***	***Sr24***	***Lr34***	***Lr37***	**Sr26**	**Sr31**	**LR response**
SST806	[redacted]								60MS
									60S
									20S
									40S

S: Susceptible

MS: Moderately susceptible

MR: Moderately resistance

TMR: Trace moderately resistant

Addendum D

Table D1. Spray inoculation of average disease severity 28 dpi.

Wheat line	Average disease severity (%)
F_6-5181	67.58
F_6-5183	73.02
F_6-5191	45.63
F_6-5192	66.57
F_6-5198	83.33
F_6-5200	43.61
F_6-5225	38.45
F_6-5243	68.33
F_6-5244	48.59
F_6-5297	38.53
F_6-5301	43.65
F_6-5397	41.28
F_6-5399	25.30
F_6-5405	50.00
F_6-5411	41.73
F_6-5426	77.14
F_6-5429	47.81
F_6-5433	50.00
F_6-5439	94.87
F_6-5440	55.00
F_6-5450	53.52
F_6-5451	34.21
F_6-5453	40.74
F_6-5523	50.00
F_6-5596	40.48
F_6-5657	50.00
F_6-5661	48.81
F_6-5704	43.96
PAN3471	79.51
Sumai3	21.15
FHB-6402	40.81
FHB-6403	42.10
FHB-6404	43.19
FHB-6405	56.64
FHB-6407	51.52
FHB-6408	53.57
FHB-6409	77.57
FHB-6410	27.08

Table D2. Point inoculation of average disease severity 28 dpi

Wheat line	Average disease severity (%)
F_6-5181	84.82
F_6-5183	88.81
F_6-5191	36.87
F_6-5192	78.46
F_6-5198	82.50
F_6-5200	34.11
F_6-5225	68.52
F_6-5243	51.19
F_6-5297	68.95
F_6-5301	88.89
F_6-5397	100.00
F_6-5399	75.56
F_6-5405	80.54
F_6-5411	61.71
F_6-5426	37.45
F_6-5429	45.40
F_6-5433	80.00
F_6-5439	63.19
F_6-5450	39.18
F_6-5451	87.70
F_6-5453	83.75
F_6-5523	100.00
F_6-5596	100.00
F_6-5636	48.72
F_6-5657	56.10
F_6-5661	56.21
F_6-5704	100.00
PAN3471	93.56
Sumai3	26.44
FHB-6402	75.56
FHB-6403	52.36
FHB-6404	100.00
FHB-6405	100.00
FHB-6407	71.10
FHB-6408	50.000
FHB-6409	75.81
FHB-6410	70.96
FHB-6411	61.83

Wheat line	Average disease severity (%)
FHB-6411	42.22
FHB-6412	44.43
FHB-6413	55.77
FHB-6414	55.56
FHB-6415	10.56
FHB-6416	44.86
FHB-6419	56.10
FHB-6420	43.33
FHB-6423	55.15
FHB-6426	45.89
FHB-6429	40.78
FHB-6430	52.27
FHB-6431	41.12
FHB-6432	51.96
FHB-6433	60.42
FHB-6434	17.86
FHB-6435	90.91

Wheat line	Average disease severity (%)
FHB-6412	95.49
FHB-6413	88.21
FHB-6414	55.77
FHB-6415	52.27
FHB-6416	83.33
FHB-6417	100.00
FHB-6419	84.58
FHB-6420	100.00
FHB-6423	100.00
FHB-6426	68.06
FHB-6429	100.00
FHB-6430	82.64
FHB-6431	94.23
FHB-6432	78.02
FHB-6433	96.30
FHB-6434	62.95
FHB-6435	47.62